Samantha Lee Lab NB

June 8th, 2015

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PCR for PTEF(m3)
    Protocol:
        Promoter PCR:
            1. Forward Primer (10uM -Diluted 10X)                    2.5 ul
            2. Reverse Primer (10uM -Diluted 10X)                    2.5 ul
            3. Water (ddH2O)                                         19.5 ul
            4. Template DNA (PTEF(m3))                               0.5 ul
            5. 2X Phusion Master Mix                                 25 ul
                                                                Total: 50 ul
        Bar 1 PCR:
            1. Primer #92 (10uM -Diluted 10X)                        2.5 ul
            2. Primer #93 (10uM -Diluted 10X)                        2.5 ul
            3. Water (ddH2O)                                         19 ul
            4. Bar 1 Template DNA                                    1 ul
            5. 2X Phusion Master Mix                                 25 ul
                                                                Total: 50 ul
        Thermocycler:
            Initial Denature                    98 degree                        30s
                35 cycles of
                    Denature                        98 degree                        10s
                    Anneal                          55 degree                        20s
                    Extension                       72 degree                        1'
            Final Extension                         72 degree                        5'
            Hold                                    4  degree                        forever
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June 9th, 2015

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Agarose Gel and Enzyme Digest
    Agarose Gel:
        40 ul of Agrose 1% gel
        4  ul of dye with gel
                        Run gel at 100 volts
            Promoter and Bar 1:
                1. Add 5 ul of either Promoter or Bar 1
                2. Add 1 ul of Tracking Dye into the tube
                        Total of 6 ul
       Run only 5 ul of promoter or Bar 1 in gel in case of pipetting errors.
            Plasmid:
                1. Add 30 ul of plasmid
                2. Add 6 ul of loading dye (1 to 5 ratio)into a tube
   Lab Errors:
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1. We mixed up our Bar 1 and Promoter tubes and had to restart
2. While loading the gel, the ladder went into our Bar 1 lane and we did had to reload another Bar 1 into the gel.

Enzyme Digest Lab (cut @ restriction sites):

Promoter Digest:

1. Add 5 ul CutSmart Buffer to your PCR tube
2. Add 0.5 Apa1 restriction enzyme to the tube.
3. Vortex to mix well.
4. Incubate @ room temperature for at least 1 hour.

STOP HERE

5. Add 0.5 ul Xho1 restriction enzyme to the tube.
6. Vortex to mix well.
7. Incubate @ 37 degree for at least 1 hour.

Bar 1 Digest:

1. Add 5 ul CutSmart Buffer to your PCR tube.
2. Add 0.5 ul Xho1 and 0.5 ul Not1 restriction enzyme to the tube.
3. Vortex to mix well
4. Incubate @ 37 degree for @ least 1 hr.

Plasmid Digest:

1. Take 10 ul of plasmid from the plasmid stock (pJW608) into a new tube.
2. Add 3 ul CutSmart Buffer and 16 ul of water to your tube.
3. Add 0.5 ul Apa1 restriction enzyme to the tube.
4. Vortex to mix well.
5. Incubate @ room temperature for @ least 1 hr.

STOP HERE

6. Add 0.5 ul Not1 restriction enzyme to the tube.

Error: I added the enzyme Xho1 instead of Not1. After realizing this, I added Not1 into the tube too.

7. Vortex to mix well.
8. Incubate @ 37 degree for @ least 1 hr.

Gel Photo and Key is in the physical lab notebook or in "Gel Photos" folder.

June 10th, 2015

Agarose Gel Extraction

1. Add loading dye to digest tubes.

Dye: 6X (1 to 5 dye ratio)

a. Promoter: Add 10 ul of dye because there's a lot of promoter
b. Bar 1: Add 10 ul of dye to large amounts of Bar 1
c. Plasmid: Add 6 ul of dye due to the 30 ul of plasmid

Extracting the gel:

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.
3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol.
5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through

7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Ligation Protocol

1. Set up the following reaction in a microcentrifuge tube on ice.

Materials:

1. Nuclease free water	8 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (4kb) ---> Promoter	4 ul
4. Insert DNA (1kb) ---> Bar 1	4 ul
5. Plasmid	1 ul
6. T4 DNA Ligase	1 ul
	Total: 20 ul

2. Gently mix the reaction by pipetting up & down and microfuge briefly
3. For COHESIVE (STICKY) ENDS, incubate @ 16 degree overnight (o/n) or room temp. for 10 min.
4. For BLUNT ENDS or SINGLE BASE OVERHANGS, incubate @ 16 degree o/n or room temp for 2 hrs.
5. Heat inactive @ 65 degree for 10 min.
6. Chill on ice & transform 5 ul of the reaction into 50 ul competent cell

Transformation Protocol

1. Add 0.5 ul of plasmid to 25 ul of competent cells
   -make sure the competent cells are thawed (10min on ice)
   -mix gently
2. Flick tube 5 times to mix the cells & DNA
3. Place on ice for 10 min
4. Heat shock for 30s @ 42'C
5. Place on ice for 5 min
6. Add 50 ul of SOC media under fire
7. Place @ 37'C for 1 hr while shaking
8. Warm LB-Carb plates
9. Plate 100 ul of cells
10. Place plates in 37'C incubater for o/n

June 11th, 2015

Colony PCR

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later.

Repeat for all 6 colonies (1 tube per 1 colony)

5. Make a "Master Mix" of PCR components (PCRing 6 colonies, make for 7)

	1x Reaction	7x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	70 ul
b. 10 uM FW primer (pTEFm3)	1 ul	7 ul
c. 10 uM REV primer (Bar 1 #93)	1 ul	7 ul
d. water	3 ul	21 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and

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spin down in the capsule microfuge.
    7. Put PCR strips in thermocycler for the  cycle (saved file "GoTaq)
                        95                      5 min
                30x:    95                      45s
                       55                       30s
                        72                      1' per kb
                72                             10'
    8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye    in it, so just directly load
 5-10 ul of your PCR reaction into gel
            a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul
    9. For all positive bands on the gel, take the rest of the bacterial cells    from step 1 & inoculate them
 into an o/n LB (+antibiotic) for miniprep.
FOR (-) CONTROL: DON'T PUT IN INSERT
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June 12th, 2015

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Miniprep
    1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
    2. Add 250 ul Buffer P1 (found in refridgerator)
    3. Transfer to a microcentrifuge tube
    4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
    5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
    6. Centrifuge for 30s. Discard the flow-through
    7. Apply the supernatants from step 6 to the spin column by pipetting
    8. Centrifuge for 30s. Discard the flow-through
    9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for        30s
    10. Discard flow-through & centrifuge @ full speed for 1 min to remove         residual wash buffer
    11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA,       add 50 ul of water to the
center of the spin column, let it stand for       1 min, & centrifuge for 1 min.
DNA Sequencing
    1. Pipette 5 ul of plasmid into 1.5 ml tubes
        Tube #1: "SL1"
        Tube #2: "SL2"
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June 15th, 2015 and June 16th, 2015

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No lab work, just lectures.
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June 17th, 2015

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Positive Feedback Notes:
            mFalpha = secrets           Ste2 = Sense
    1. Put the new promoter (LexAOps) into the plasmid (HY86E3)
        a. Make primers (Apa1 & Xho1)
        b. PCR new promoter w/ Apa1 & Xho1 cut sites
        c. Digest PCR & plasmid w/ Apa1 & Xho1
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        d. Ligate together
        e. Transform into bacteria (E. Coli, the LB + Carb plates)
    2. PCR mFalpha & Ste2 w/ Xho1 & Not1 cut sites
        a. Digest
        b. Ligate
    3. Put into the yeast
    4. Combine positive feedbacks (mFalpha & Ste2)
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June 22nd, 2015

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Resconstituted (Rehydrating) the Primers (Apa1 & Xho1):
    Forward Primer (Apa1): 35.2 nMol
        35.2 nMol x 10 = 352 ul
            Add 352 ul of water (ddH2O) into the primer tube
    Reverse Primer (Xho1): 29.9 nMol
        29.9 nMol x 10 = 299 ul
            Add 299 ul of water into the primer tube
Dilute the Forward (FW) and Reverse (RV) primers:
    FW Primer: 10 ul of FW Primer + 90 ul of water
    RV Primer: 10 ul of RV Primer + 90 ul of water
            Leftovers in working stock box
PCR the Constitutive Promoter #1:
    1. Forward Primer (Apa1)(10uM -Diluted 10X)                  2.5 ul
    2. Reverse Primer (Xho1) (10uM -Diluted 10X)                 2.5 ul
    3. Water (ddH2O)                                            19.5 ul
    4. Template DNA (T64-M64)                                    0.5 ul
    5. 2X Phusion Master Mix                                      25 ul
                                                          Total: 50 ul
Thermocycler Protocol:
            Initial Denaturation           98'C               30s
            35 Cycles
                -Denaturation                98'C             10s
                -Annealing                  55'C               20s
                -Extension                  72'C             30s
            Final Extension                 72'C               5m
            Hold                           4'C             Forever
1% Agarose Gel
    Test if the PCR worked by putting it on an agarose gel
        1. Pour 35 mL of Agarose into a falcon tube
        2. Add 8.5 ul (should be 5ul)of SybeSafe to the gel mixture
        3. Add 5 ul of FW and RV Primer into a new microcentrifuge tube
        4. Add 1 ul of tracking dye to DNA (no SDS)
    Gel Photo is in physical lab notebook & box titled as "LexAOps (+ApaI +       XhoI) PCR #1"
        Error: This gel showed our band but it was not bright so we did                    another PCR to
amplify it so that the band will be brighter and              we can digest it.
PCR Promoter #2:
    To increase amount of the promoter we have
        1. In a microcentrifuge tube, add 2.5 ul of FW primer
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2. Add 2.5 ul of RV primer
3. Add 18 ul of water (ddH2O)
4. Add 2 ul of DNA template (liquid in last PCR tube)

Gel Photo is in physical lab notebook & box titled as "LexAOps (+ApaI + XhoI) PCR #2 30 mins" because we ran this gel for 30 mins.

Error: The bands on the gel was too clustered so we ran the gel for another 15 mins. Please refer to the gel "LexAOps (+ApaI + XhoI) PCR #2 45 mins".

Culturing Plasmid T-64 (td Tomato)

Culturing this plasmid was to help the Basic Circuit members

Materials:

1. 1 liquid culturing tube (falcon tube)
2. 1 tooth pick
3. 5 mL of LB + Carb media
4. Bunsen Burner

Protocol:

1. Label the culturing tube
2. Pour 5 mL of LB + Carb media into the falcon tube under flame
3. Use a pick & get ONE colony
4. Stir in falcon tube
5. Incubate o/n

June 23rd, 2015

Promoter and Plasmid Digest, Transformation of Hy86E3 and pGEM38, Made TAE Buffer and Agarose Gel, PCR Promoter #3, and Gel Extraction

Promoter Digest:

1. Add 5 ul of CutSmart Buffer to PCR tube
2. Add 0.5 ul of Apa1 restriction enzyme to tube
3. Vortex
4. Incubate @ room temp. for 1 hr
5. Add 0.5 ul of Xho1 restriction enzyme to tube
6. Vortex
7. Incubate @ 37'C for 1 hr

Plasmid (HY83E4) Digest:

1. Take 10 ul of plasmid into a new PCR tube
2. Add 3 ul of CutSmart Buffer
3. Add 16 ul of water (ddH2O)
4. Add 0.5 ul of Apa1 restriction enzyme
5. Vortex
6. Incubate @ room temp. for 1 hr
7. Add 0.5 ul of Not1 restriction enzyme
8. Vortex
9. Incubate @ 37'C for 1 hr

Error: The plasmid (HY83E4) was the wrong plasmid, now we have to redo the digest step with the plasmid (HY86E3)

Place the promoter & plasmid digest in a 1% agarose gel

1. Poured a small gel of 25 mL Agarose gel and 2.5 ul of Sybersafe dye
   -Sybersafe is 1/10 in ul

Gel Photo is in physical lab notebook and in box under "LexAOps + Hy86E3 Digestion"

Error: In lane 4 and 5 are our promoter (Apa1 + LexA Ops + Xho1) but it doesn't appear in our gel, meaning that our digest for the promoter did not work. However, our plasmid (Hy86E3) appears so we did a gel extraction of that.

Transform HY86E3 and pGEM38

We were running low on HY86E3 plasmid so we transformed it into bacteria (E.coli) to make more while waiting for our digest to finish incubating.

1. Add 0.5 ul of plasmid HY86E3 into a new microcentrifuge tube
2. Add 25 ul of competent cells (make sure it's thawed on ice then mix gently)
3. Flick the tube 5 times to mix the cells & DNA
4. Place on ice for 10 mins
5. Heat Shock for 30s @ 42'C
6. Place on ice for 5 min
7. Add 450 ul of SOC media under a flame
8. Place @ 37'C for 1 hr while shaking
9. Warm LB -Carb plates
10. Plate 100 ul of cells
11. Place plates in 37'C incubater o/n

Transform pGEM38

We were running low on pGEM plasmid so we transformed it into bacteria (E.coli) to make more while waiting for our digest to finish incubating.

1. Add 0.5 ul of plasmid HY86E3 into a new microcentrifuge tube
2. Add 25 ul of competent cells (make sure it's thawed on ice then mix gently)
3. Flick the tube 5 times to mix the cells & DNA
4. Place on ice for 10 mins
5. Heat Shock for 30s @ 42'C
6. Place on ice for 5 min
7. Add 450 ul of SOC media under a flame
8. Place @ 37'C for 1 hr while shaking
9. Warm LB -Carb plates
10. Plate 100 ul of cells
11. Place plates in 37'C incubater o/n

Making 1X TAE Buffer

1. Use a 900 mL flask (near the freezer room with the plates)
2. Poor 900 mL of "Milli-Q water" (distilled water) into the flask
3. Pour 80 mL more of "Milli-Q water" into a graduated cylinder, then into the flask
4. Pour 20 mL of 50X TAE Buffer
    a. 1 mL of TAE Buffer to 49 mL of Milli-Q water
5. Mix by swirling

Making 1% Agarose Gel

1. Use a 500 mL flask
2. Pour 500 mL of TAE Buffer
3. Weigh 5g of Agarose Powder
    a. 1% Agarose Gel = 1g of Agarose Powder
4. Microwave on potato (3 mins) until clear but mix when it bubbles.

PCR the Promoter #3:

We decided to switch it up and did two experiments with this, one reaction had DMSO and the other one did not have DMSO.

1. Forward Primer (Apa1)(10uM -Diluted 10X) 2.5 ul
2. Reverse Primer (Xho1) (10uM -Diluted 10X) 2.5 ul
3. Water (ddH2O) 19.5 ul
4. Template DNA (T64-M64) 0.5 ul
5. 2X Phusion Master Mix 25 ul

Total: 50 ul

1. Forward Primer (Apa1)(10uM -Diluted 10X) 2.5 ul
2. Reverse Primer (Xho1) (10uM -Diluted 10X) 2.5 ul
3. Water (ddH2O) 18 ul
4. Template DNA (T64-M64) 0.5 ul
5. DMSO 1.5 ul
5. GoTaq Green Master Mix 25 ul

Total: 50 ul

Thermocycler Protocol:

Initial Denaturation	95'C	2m
5 Cycles		
-Denaturation	95'C	30s
-Annealing	45'C	30s
-Extension	72'C	1m
30 Cycles		
	95'C	30s
	55'C	30s
	72'C	1m
Final Extension	72'C	1m
Hold	4'C	Forever

Gel Extraction for plasmid (Hy86E3)

Extracting the gel:

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.
    a. Gel weighed about 107 mg
    b. We added 320 ul instead of 321 ul of Buffer QG
3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol
    a. Added 107 ul of isopropanol
5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nano-drop:

After a gel extraction protocol, always nano-drop it to find the concentration of DNA in it.

1. Wipe down machine
2. Blank it with water (ddH2O)
3. Place 1.5 ul into the machine
4. Write down on tube the number received
    a. Result: Plasmid Gel Extract was 9.839 ng/ul

June 24th, 2015

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Touchdown PCR & Digest Promoter (Apa1 + LexA Ops + Xho1)
    We switched it up by using a different DNA Template PCR protocol, and DNA   polymerase.
        LexA Ops Promoter PCR #4:
            1. 2.5 ul of FW Primer
            2. 2.5 ul of RV Primer
            3. 0.5 ul DNA Template T-64 (td Tomoato)
            4. 25 ul of Phusion Master Mix
            5. 19.5 ul of Water (ddH2O)
        LexA Ops Promoter PCR #4:
            1. 2.5 ul of FW Primer
            2. 2.5 ul of RV Primer
            3. 0.5 ul DNA Template T-64 (td Tomoato)
            4. 25 ul of GoTaq Green Master Mix
            5. 19.5 ul of Water (ddH2O)
        Touchdown PCR Protocol General:
                1. 95'C                     2m
            Repeat 10x
                2. 95'C                     30s
                3. 72'C                     30s
                    -1'C per cycle
                4. 72'C                     1min 30s
            Repeat 36x
                5. 95'C                     30s
                6. 55'C                     30s
                7. 72'C                     1min 30s
                8. 76'C                     3m
                9. 10'C or 4'C              Forever
        Touchdown PCR Protocol Phusion:
                Initial Denature                98'C                30s
                10 cycles of
                    Denature                    98'C                10s
                    Anneal                  55'C                20s
                        -1'C per cycle
                    Extenstion              72'C                30s
                30 cycles of
                    Denature                    98'C                10s
                    Anneal                  55'C                20s
                    Extension               72'C                30s
                Final Extension             72'C                5m
                Hold                            4'C             Forever
        Touchdown PCR Protocol GoTaq:
                Initial Denature                95'C                2m
                10 cycles of
                    Denature                    95'C                30s
                    Anneal                  55'C                30s
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-1'C per cycle
Extenstion 72'C 1m
30 cycles of
Denature 95'C 30s
Anneal 55'C 30s
Extension 72'C 1m
Final Extension 72'C 5m
Hold 4'C Forever

Gel Loading

Placed our Phusion and GoTaq PCR on a gel to ensure our promoter is present.

Phusion PCR:

1. Add 5 ul of "PPCR" (our PCR) into a new tube
2. Add 1 ul of loading dye (purple gel loading dye 6x)

Gel is found in physical notebook and in box under "LexAOps (+ApaI + XhoI) PCR #4"

1. A band was found at 600bp for the Phusion sample
2. No bands were found for the GoTaq sample

June 25th, 2015

Promoter Digest, Miniprep, Gel Extraction, Ligation, Transformation

LexAOps + Apa1 + Xho1 Digest

1. Add 5 ul of CutSmart Buffer to PCR tube
2. Add 0.5 ul of Apa1 restriction enzyme to tube
3. Vortex to mix well
4. Incubate @ room temp. for 1 hr
5. Add 0.5 ul of Xho1 restriction enzyme to tube
6. Vortex
7. Incubate @ room temp. for 1 hr

Miniprep

1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

Made 1.5% Agarose Gel

100 mL of 1.5% Agarose Gel:

1. Pour 100 mL of 1x TAE Buffer into a 500 mL flask
2. Weigh 1.50g of Agarose Powder
3. Microwave & mix
4. Add 3.5 ul of Sybersafe (1/10) once 35 mL is poured into a falcon tube

Sybersafe is 10,000x

35 mL Gel = 3,500 = 3.5 ul

Loading Gel w/Phusion PCR:

Add loading dye to LexAOps + Apa1 + Xho1 Digest (51 ul total)

1. Add 10 ul of gel loading dye (6x)

35 mL Gel = 3,500 = 3.5 ul

60 ul (total)/ 6x = 10 ul

-Used a 1.5% Agarose Gel because the promoter size is 665bp long so we need a denser gel to separate the bands better.

-Gel photo in box under "LexAOps Digestion #2"

Gel Extraction for Promoter (LexAOps)

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube (152 mg). Add Buffer QG (3 times the volume of the gel) to the tube (460 ul).
3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol (152 ul).
5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nanodrop: 7.665 ng/ul

Ligation Protocol:

1. Set up the following reaction in a microcentrifuge tube on ice.

Materials for plasmid (Hy86E3) & Promoter (LexAOps):

1. Nuclease free water	8 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (4kb) ---> Plasmid (Hy86E3)	4 ul
4. Insert DNA (1kb) ---> Promoter(LexAOps)	4 ul
5. Plasmid	1 ul
6. T4 DNA Ligase	1 ul
	Total: 20 ul

Materials for (-) control:

1. Nuclease free water	11 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (4kb) ---> Plasmid (Hy86E3)	6 ul
4. T4 DNA Ligase	1 ul
	Total: 20 ul

Materials for plasmid (Hy86E3)Ligation & Promoter (LexAOps)-Experiment:

1. Nuclease free water	9 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (4kb) ---> Promoter (Hy86E3) 55ng	6 ul
4. Insert DNA (1kb) ---> (665bp) 15ng	4 ul
6. T4 DNA Ligase	1 ul
	Total: 20 ul

2. Gently mix the reaction by pipetting up & down and microfuge briefly
3. For COHESIVE (STICKY) ENDS, incubate @ 16 degree overnight (o/n) or room temp. for 10 min.
4. For BLUNT ENDS or SINGLE BASE OVERHANGS, incubate @ 16 degree o/n or room temp for 2 hrs.
5. Heat inactive @ 65 degree for 10 min.

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    6. Chill on ice & transform 5 ul of the reaction into 50 ul competent cell
Transform LexAOps & Hy86E3
    1. Add 0.5 ul of plasmid (LexAOps & Hy86E3) to 25 ul of competent cells
        -make sure the competent cells are thawed (10min on ice)
        -mix gently
    2. Flick tube 5 times to mix the cells & DNA
    3. Place on ice for 10 min
    4. Heat shock for 30s @ 42'C
    5. Place on ice for 5 min
    6. Add 50 ul of SOC media under fire
    7. Place @ 37'C for 1 hr while shaking
    8. Warm LB-Carb plates
    9. Plate 100 ul of cells
    10. Place plates in 37'C incubater for o/n
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June 26th, 2015

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Colony PCR for plasmid Hy86E3 & promoter LexAOps
        1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6          PCR tubes.
        2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
        3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that            colony. Set pipette to 5
 ul and pipette up & down to mix in 25 ul             of water.
        4. Transfer 5 ul of this into your empty PCR tubes. Save remaining            20 ul for later.
                Repeat for all 6 colonies (1 tube per 1 colony)
     5. Make a "Master Mix" of PCR components (PCR 6 colonies, make for 7)
                                        1x Reaction     7x Reaction Master
         a. 2x GoTaq Green PCR Mix      10 ul               130 ul
            b. 10 uM FW primer (pTEFm3)     1 ul                13 ul
         c. 10 uM REV primer (Bar 1 #93)    1 ul                13 ul
            d. water                           3 ul             39 ul
                                                            total: 195 ul
     6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x             Master Mix. Vortex to mix
and spin down in the capsule microfuge.
        7. Put PCR strips in thermocycler for the  cycle (saved file "GoTaq)
                           95                       5 min
                    30x:   95                       45s
                        55                     30s
                         72                        1' per kb
                           72                     10'
     8. Analyze products on a 1% agarose gel. GoTaq mix already has loading          dye in it, so just
directly load 5-10 ul of your PCR reaction into         gel
            a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul
    9. For all positive bands on the gel, take the rest of the bacterial cells    from step 1 & inoculate them
 into an o/n LB (+antibiotic) for miniprep.
FOR (-) CONTROL: DON'T PUT IN INSERT
Colony PCR:
    (+) Should amplify the insert DNA (LexAOps)
    (-) Doesn't have insert so nothing to PCR/Amplify (just has cut sites)
```

```
Gel test:
    1.5% Agarose gel
        (+) should show because it has cut sites & LexAOps
        (-) should be a small band because only has cut sites so the band will         run off the gel.
        -12 PCR tubes for experiment (6 of them are for PCR) ---> 5 ul
     -4 PCR tubes for (-) control (2 of them for PCR) ---> 5 ul
    Touchdown PCR Protocol GoTaq:
                Initial Denature                95'C                5m
                10 cycles of
                    Denature                    95'C                45s
                    Anneal                  55'C                30s
                        -1'C per cycle
                    Extenstion              72'C                1m
                30 cycles of
                    Denature                    95'C                30s
                    Anneal                  55'C                30s
                    Extension               72'C                1m
                Final Extension (lower temp. because DNA is longer) 10'C    5m
                Hold                            4'C             Forever
        Gel photo in physical lab notebook and in box under "LexAOps + Hy86E3       Colony PCR"
            Error: Lane 7 only had 6 ul to load but it had the green color so                it may not have
 any cells.
Liquid Culturing for Plasmid pNH605 (Hy86E3) and Promoter LexAOps
    Under a flame:
        1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
        2. Add 20 ul of our colony to PCR tubes into falcon tubes
        3. Vortex and 4'C fridge o/n
        4. Place in 37'C incubator o/n
```

June 29th, 2015

```
Placed the liquid cultures of LexAOps + Hy86E3 in the incubator all day.
```

June 30th, 2015

```
Miniprep LexAOps and Hy86E3 plasmid liquid cultures & DNA Sequence
    Miniprep LexAOps and Hy86E3 plasmids (#1-5):
        1. Centrifuge liquid cultures for 10 min
        2. Resuspend cells in 250 ul Buffer P1
        3. Transfer mixtures to 1.5 mL tubes
        4. Add 250 ul Buffer P2. Mix by inverting 6x
        5. Add 350 ul Buffer N3. Mix by inverting 6x
        6. Centrifuge for 10 mins
        7. Pour supernatant into QIAprep spin column
        8. Centrifuge for 30s & discard flow through
        9. Add 0.75 mL Buffer PE to column to wash. Centrifuge for 30s
        10. Discard flow-through. Centrifuge for 1 min
```

```
        11. Place column in new 1.5 mL tube
        12. Add 50 ul of water to column. Let it stand for 1 min
        13. Centrifuge for 1 min
    Nanodrop:
        Measured 1.5 ul of each tube
            -LexAOps + Hy86E3 Experiment #1     517.6 ng/ul
            -LexAOps + Hy86E3 Experiment #2     746.5 ng/ul
            -LexAOps + Hy86E3 Experiment #3     252.8 ng/ul
            -LexAOps + Hy86E3 Experiment #4     204.7 ng/ul
            -LexAOps + Hy86E3 Experiment #5     172.9 ng/ul
    DNA Sequencing:
        Sent Experiment samples #3-#5 to Quintara for DNA sequencing.
```

July 1st, 2015

```
DNA Sequencing failed and now we have to redo our PCR and digestion from the  beginning.
    LexAOps Promoter PCR #5
        LexAOps Promoter PCR #5 (Td-Tomato):
            1. 10uM FW Primer-129 (+ApaI)     2.5uL
            2. 10uM RV Primer-130 (+XhoI)     2.5uL
            3. T-64 (tdTomato) Template DNA   0.5uL
            4. 2x Phusion Master Mix          25.0uL
            5. Water                          19.5uL
                                Total:      50.0uL
        LexAOps Promoter PCR #5 (T-64 M-64):
            1. 10uM FW Primer-129 (+ApaI)     2.5uL
            2. 10uM RV Primer-130 (+XhoI)     2.5uL
            3. T-64 M-64 Template DNA         0.5uL
            4. 2x Phusion Master Mix          25.0uL
            5. Water                          19.5uL
                                Total:      50.0uL
            Thermocycler Protocol:
                TouchDownPhusion
                    Initial Denaturation       98'C    30s
                    10 Cycles
                        Denaturation       98'C    10s
                        Annealing          55'C    20s
                    (-1'C Per Cycle)
                        Extension          72'C    30s
                    30 Cycles
                        Denaturation       98'C    10s
                        Annealing          55'C    20s
                        Extension          72'C    30s
                        Final Extension        72'C    5m
                    Hold                   4'C      Forever
        Gel Loading:
            1. 1.5% Agarose Gel   35uL
            2. SyberSafe          3.5uL
```

Key:

2-Log DNA Ladder	10.0uL	Lane #1
LexAOps Promoter (Td-Tomato)	6.0uL	Lane #2
LexAOps Promoter (T-64 M-64)	6.0uL	Lane #3

Gel Results:

1. See Gel Folder for gel labeled "LexAOps PCR #5 30/40mins"
2. Bands visible,but appears closer to 600bp than expected 665bp

LexAOps & Hy86E3 & Hy111E2 Digestion

LexAOps (Td-Tomato) Digestion:

1. Added 5.0uL of CutSmart Buffer
2. Added 0.5uL of ApaI
3. Vortexed
4. Incubated at room temperature for 1 hour
5. Added 0.5uL XhoI
6. Vortexed
7. Incubated at 37'C overnight

LexAOps (T-64 M-64) Digestion:

1. Added 5.0uL of CutSmart Buffer
2. Added 0.5uL of ApaI
3. Vortexed
4. Incubated at room temperature for 1 hour
5. Added 0.5uL XhoI
6. Vortexed
7. Incubated at 37'C overnight

Hy86E3 Digestion:

1. Transferred 10.0uL of plasmid into a new PCR tube
2. Added 16.0uL of water
3. Added 3.0uL of CutSmart Buffer
4. Added 0.5uL of ApaI
5. Vortexed
6. Incubated at room temperature for 1 hour
7. Added 0.5uL of XhoI
8. Vortexed
9. Incubated at 37'C overnight

Hy111E2 Digestion:

1. Transferred 10.0uL of plasmid into a new PCR tube
2. Added 16.0uL of water
3. Added 3.0uL of CutSmart Buffer
4. Added 0.5uL of ApaI
5. Vortexed
6. Incubated at room temperature for 1 hour
7. Added 0.5uL of XhoI
8. Vortexed
9. Incubated at 37'C overnight

July 2nd, 2015

Ran digestion on a gel, gel extraction for digest, PCR mFα and Ste2 genes, and ligated LexAOps with plasmid &

transformed into E.coli.

LexAOps & Hy86E3 & Hy111E2 Digestion Part 2

Gel Loading:

1% Agarose Gel 35 uL

SyberSafe 3.5 uL

Key:

2-Log DNA Ladder	10.0uL	Lane #1
LexAOps Promoter (Td-Tomato)	30.0uL	Lane #2
LexAOps Promoter (Td-Tomato)	30.0uL	Lane #3
LexAOps Promoter (T-64 M-64)	30.0uL	Lane #4
LexAOps Promoter (T-64 M-64)	30.0uL	Lane #5
Hy111E2 Plasmid	36.0uL	Lane #6
Hy86E3 Plasmid	36.0uL	Lane #7
2-Log DNA Ladder	10.0uL	Lane #8

Gel Results:

-See Gel Folder for gel labeled "LexAOps (Td-Tomato + M-64) + Hy111E2 + Hy86E3 30/40mins"

-Bands visible, but no bands for Hy111E2 plasmid

-LexAOps (Td + M-64) + Hy86E3 Digestion Gel Extration:

-Excised gel bands in lanes 2, 3, 4, 5, and 7 and transferred to 1.5mL tube

Extracting the gel:

1. Using a clean,sharp blade,cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.

Weight of gel slices:

-LexAOps (Td-Tomato): 153.0mg

-LexAOps (T-64 M-64): 140.0mg

-Hy86E3 Plasmid: 158.0mg

Buffer QG:

-Added Buffer QG in a 3:1 ratio (100mg ~ 100uL)

-LexAOps (Td-Tomato): +480.0uL

-LexAOps (T-64 M-64): +420.0uL

-Hy86E3 Plasmid: +470.0uL

3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol

-Added isopropanol in a 1:1 ratio

-LexAOps (Td-Tomato): +153.0uL

-LexAOps (T-64 M-64): +140.0uL

-Hy86E3 Plasmid: +158.0uL

5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash:add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nano-Drop:

Tested 1.5uL each sample

Results:

LexAOps (Td-Tomato): 6.259ng/uL
LexAOps (T-64 M-64): 8.964ng/uL
Hy86E3 Plasmid: 46.22ng/uL

Ligation Protocol for LexAOps (Td & M64) + Hy86E3:

LexAOps (Td-Tomato) + Hy86E3 Ligation:

1. 10x T4 DNA Ligase Buffer: 2.0uL
2. Hy86E3 DNA (55ng): 1.2uL
3. LexAOps DNA (15ng): 2.4uL
4. Nuclease-Free Water: 13.4uL
5. T4 DNA Ligase: 1.0uL

Total: 20.0uL

6. Mixed reaction by pipetting up and down
7. Incubated at room temperature for 1 hour

LexAOps (T-64 M-64) + Hy86E3 Ligation:

1. 10x T4 DNA Ligase Buffer: 2.0uL
2. Hy86E3 DNA (55ng): 1.2uL
3. LexAOps DNA (15ng): 1.8uL
4. Nuclease-Free Water: 14.0uL
5. T4 DNA Ligase: 1.0uL

Total: 20.0uL

6. Mixed reaction by pipetting up and down
7. Incubated at room temperature for 1 hour

Negative Control:

1. 10x T4 DNA Ligase Buffer: 2.0uL
2. Hy86E3 DNA (55ng): 1.2uL
3. Nuclease-Free Water: 15.8uL
4. T4 DNA Ligase: 1.0uL

Total: 20.0uL

5. Mixed reaction by pipetting up and down
6. Incubated at room temperature for 1 hour

LexAOps (Td + M-64) + Hy86E3 Transformation

LexAOps T64 (Td-Tomato) + Hy86E3 Transformation:

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 5uL of ligased LexAOps (Td-Tomato) + Hy86E3 to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate

15. Incubated both plates over-weekend at room temperature

LexAOps (T-64 M-64) + Hy86E3 Transformation:

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 5uL of ligased LexAOps (T-64 M-64) + Hy86E3 to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over-weekend at room temperature

Negative Control Transformation:

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 5uL of negative control sample to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over-weekend at room temperature

mFa + Ste2 PCR

Reconstitute Primers:

mFa (+LexAOps) FW-131:

31.0 nmoles x 10 = 310uL

mFa (+pNH605) RV-132:

31.3 nmoles x 10 = 313uL

Ste2 (+LexAOps) FW-133:

32.6 nmoles x 10 = 326uL

Ste2 (+pNH605) RV-134:

28.2 nmoles x 10 = 282uL

Dilution:

10uL primers + 90uL water = 10uM stock solution / 100uL

```
            mFa PCR:
                10uM FW Primer-131 (+LexAOps)   2.5uL
                10uM RV Primer-132  (+pNH605)   2.5uL
                pTS133 Template DNA             0.5uL
                2x Phusion Master Mix           25.0uL
                Water                           19.5uL
                                    Total:      50.0uL
            Ste2 PCR:
                10uM FW Primer-133 (+LexAOps)   2.5uL
                10uM RV Primer-134  (+pNH605)   2.5uL
                *Ste2(?) Template DNA           0.5uL
                2x Phusion Master Mix           25.0uL
                Water                           19.5uL
                                    Total:      50.0uL
                        *Unknown whether contained Ste2 gene or not
                    Thermocycler Protocol:
                        Initial Denaturation        98'C    30s
                        35 Cycles
                            Denaturation            98'C    10s
                            Annealing               55'C    20s
                            Extension               72'C    45s
                        Final Extension         72'C    5m
                        Hold                        4'C     Forever
        Gel Loading:
            1.5% Agarose Gel        35uL
            SyberSafe               3.5uL
    Key:
        2-log DNA Ladder            10uL    Lane #1
        mFa PCR                 6.0uL   Lane #2
        Ste2 PCR                    6.0uL   Lane #3
            Gel Results:
                -See Gel Folder for gel labeled "mFa + Ste2 PCR"
                -Good Ste2 Band
                -mFa Band faint. Attempt to reamplify with second PCR.
```

July 6th, 2015

```
Colony PCR LexAOps & Hy86E3 #2
    1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR      tubes.
    2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
    3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette
 up & down to mix in 25 ul of water.
    4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul        for later.
    Repeat for all 6 colonies (1 tube per 1 colony)
    5. Make a "Master Mix" of PCR components (PCRing 6 colonies, make for 7)
                                    1x Reaction     19x Reaction Master
        a. 2x GoTaq Green PCR Mix       10 ul               190 ul
        b. 10 uM FW primer (pTEFm3)     1 ul                19 ul
```

c. 10 uM REV primer (Bar 1 #93) 1 ul 19 ul

d. water 3 ul 57 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.

7. Put PCR strips in thermocycler for the cycle (saved file "GoTaq)

	95	5 min
30x:	95	45s
	55	30s
	72	1' per kb
72		10'

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel

a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul

9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Colony PCR Gel

1.5% Agarose Gel (100 ml) with 10 ul of Sybersafe

Ran this gel @ 100 volts for 35 mins

Key:

1. 2 Log DNA Ladder 10 ul
2. Colony 1 T64-M64 5 ul
3. Colony 2 T64-M64 5 ul
4. Colony 3 T64-M64 5 ul
5. Colony 4 T64-M64 5 ul
6. Colony 5 T64-M64 5 ul
7. Colony 6 T64-M64 5 ul
8. 2 Log DNA Ladder 10 ul
9. Colony 1 T64 (Td Tomato) 5 ul
10. Colony 2 T64 (Td Tomato) 5 ul
11. Colony 3 T64 (Td Tomato) 5 ul
12. Colony 4 T64 (Td Tomato) 5 ul
13. Colony 5 T64 (Td Tomato) 5 ul
14. Colony 6 T64 (Td Tomato) 5 ul
15. 2 Log DNA Ladder 10 ul
16. (-) Control Colony 1 5 ul
17. (-) Control Colony 2 5 ul

Please See Box for gel photo under "LexAOps + Hy86E3 Colony PCR #2"

The gel showed that our Colony PCR was unsucessful from digestion or ligation.

mFa PCR

We redid the mFa PCR, 1 using the old reaction and 1 restarted as backup.

"Re-mFa" is the one we restarted using PTS 133 for our DNA Template

"mFa 2" is the one where we took our last reaction sample as our DNA Template, since we may have mFa but just at a low amount so we tried to amplify it incase it was there.

Liquid Culture Hy86E3

4 Colonies of T64-M64 and 4 from T64 (Td-Tomato) all from the 8X plates.

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)

2. Add 20 ul of our colony to PCR tubes into falcon tubes
3. Vortex and 4'C fridge o/n
4. Place in 37'C incubator o/n

Transformation #2 of Hy86E3

1. Add 0.5 ul of plasmid to 25 ul of competent cells
   -make sure the competent cells are thawed (10min on ice)
   -mix gently
2. Flick tube 5 times to mix the cells & DNA
3. Place on ice for 10 min
4. Heat shock for 30s @ 42'C
5. Place on ice for 5 min
6. Add 50 ul of SOC media under fire
7. Place @ 37'C for 1 hr while shaking
8. Warm LB-Carb plates
9. Plate 100 ul of cells
10. Place plates in 37'C incubater for o/n

Phosphotase Treat the Digested pNH605 (Hy86E3) plasmid before Ligation

1. Add 1 ul of phosphotase into the tube
2. Incubate @ 37'C for 1 hour
3. Incubate @ 65'C for 10 mins

Ligation of T64-M64 and T64 (Td Tomato)

T64-M64

1. Set up the following reaction in a microcentrifuge tube on ice.

Materials:

T64-M64:

1. Nuclease free water	14 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (55ng) ---> Hy86E3	1.2 ul
4. Insert DNA (15ng) ---> Promoter	1.8 ul
5. T4 DNA Ligase	1 ul
	Total: 20 ul

T64 (Td-Tomato):

1. Nuclease free water	13.4 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (55ng) ---> Hy86E3	1.2 ul
4. Insert DNA (15ng) ---> Promoter	2.4 ul
5. T4 DNA Ligase	1 ul
	Total: 20 ul

(-) Control:

1. Nuclease free water	15.8 ul
2. 10x T4 DNA Ligase Buffer	2 ul
3. Vector DNA (55ng) ---> Hy86E3	1.2 ul
4. Insert DNA (15ng) ---> Promoter	0 ul
5. T4 DNA Ligase	1 ul
	Total: 20 ul

(+) Control:

1. Nuclease free water	13.8 ul
2. 10x T4 DNA Ligase Buffer	2 ul

3. Vector DNA (55ng) ---> Hy86E3 1.2 ul
4. Insert DNA (15ng) ---> Promoter 2 ul
5. T4 DNA Ligase 1 ul
Total: 20 ul
2. Gently mix by pipetting up & down and microfuge briefly
3. For COHESIVE (STICKY) ENDS, incubate @ 16 degree overnight (o/n) or room temp. for 10 min.
4. For BLUNT ENDS or SINGLE BASE OVERHANGS, incubate @ 16 degree o/n or room temp for 2 hrs.
5. Heat inactive @ 65 degree for 10 min.
6. Chill on ice & transform 5 ul of the reaction into 50 ul competent cell

Transformation #3 as backup

1. Add 0.5 ul of plasmid to 25 ul of competent cells
-make sure the competent cells are thawed (10min on ice)
-mix gently
Plasmids:
a. T64-M64
b. T64 (Td-Tomato)
c. Old Digest
d. (-) Control
2. Flick tube 5 times to mix the cells & DNA
3. Place on ice for 10 min
4. Heat shock for 30s @ 42'C
5. Place on ice for 5 min
6. Add 50 ul of SOC media under fire
7. Place @ 37'C for 1 hr while shaking
8. Warm LB-Carb plates
9. Plate 100 ul of cells
10. Place plates in 37'C incubater for o/n

July 7th, 2015

To Do:

1. Check Plates from Transformation #3
2. Colony PCR of Transformation #3
3. Liquid Culture for Transformation #3
4. Miniprep Liquid Culture
5. Run mFalpha ("Re-mFa" and "mFa 2") on a gel (1.5%) it's near 500bp

Miniprep T64 (Td-Tomato) #1,3,4

1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the

center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

Nanodrop:

1. T64 (Td-Tomato) + Hy86E3 786.3 ng/ul
3. T64 (Td-Tomato) + Hy86E3 554.1 ng/ul
4. T64 (Td-Tomato) + Hy86E3 744.9 ng/ul

Re-mFa and mFa 2 Gel:

Ran a 1.5% gel with 35 mL with 3.5 ul of Sybersafe

Key:

1. 2 Log DNA Ladder
2. Ladder Leaked
3. mFa #2
4. Re-mFa

Please see box for gel photos "mFα PCR #2 Edit" and "mFα PCR #3 Edit"

We ran it for a second time w/same samples because old TAE buffer in the gel box was making our bands streak.

PCR Miniprep (plasmid):

1. FW primer		2.5 ul
2. RV primer		2.5 ul
3. DNA Template (T64 Td-Tomato)	0.5 ul	
4. 2X Phusion Master Mix		25 ul
5. Water		19.5 ul
	Total:	50 ul

Thermocycler Protocol:

TouchDownPhusion

Initial Denaturation	98'C	30s
10 Cycles		
Denaturation	98'C	10s
Annealing	55'C	20s
(-1'C Per Cycle)		
Extension	72'C	30s
30 Cycles		
Denaturation	98'C	10s
Annealing	55'C	20s
Extension	72'C	30s
Final Extension	72'C	5m
Hold	4'C	Forever

Colony PCR (GoTaq) #4:

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later.

Repeat for all 6 colonies (1 tube per 1 colony)

5. Make a "Master Mix" of PCR components (PCRing 6 colonies, make for 7)

	1x Reaction	21x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	210 ul
b. 10 uM FW primer (#129 LexAOps + Apa1)	1 ul	21 ul
c. 10 uM REV primer (#130 LexAOps + Xho1)	1 ul	21 ul

d. water 3 ul 63 ul

Total: 315 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.

7. Put PCR strips in thermocycler for the cycle (saved file "GoTaq")

	95	5 min
30x:	95	45s
	55	30s
	72	1' per kb
	72	10'

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel

a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul

9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Made 1.5% Agarose Gel @ 400 mL volume

1. Pour 400 mL of 1X TAE Buffer into a 500 mL flask
2. Weigh 6g of Agarose Powder

Colony PCR & Miniprep Gel:

Poured 100 mL of 1.5% Agarose Gel with 10 ul of Sybersafe

Key:

1. Loading dye (Erik accidently added)
2. 2 Log DNA Ladder
+3. T64 Td Tomato #1 Miniprep DNA
+4. T64 Td Tomato #3 Miniprep DNA
+5. T64 Td Tomato #4 Miniprep DNA
6. T64-M64 #1 Colony PCR
7. T64-M64 #2 Colony PCR
*8. T64-M64 #3 Colony PCR
9. T64-M64 #4 Colony PCR
10. T64-M64 #5 Colony PCR
11. T64-M64 #6 Colony PCR
12. (-) Control #1 Colony PCR
13. (-) Control #2 Colony PCR
*14. Old Digest (OD) #1 Colony PCR
*15. Old Digest (OD) #2 Colony PCR
16. Old Digest (OD) #3 Colony PCR
17. Old Digest (OD) #4 Colony PCR
18. Old Digest (OD) #5 Colony PCR
19. Error in our PCR tubes there wasn't anything for colony #6
20. 2 Log DNA Ladder

Please see box for gel photo called "LexAOps + Hy86E3 Colony PCR #3"

The "+" were sent to DNA Sequencing and the "*" was taken for a liquid culture.

DNA Sequencing Results:

Our plasmid didn't digest so our pTET is between our 2 cut sites (Apa1 & Xho1) instead of our promoter LexAOps.

Did our LexAOps even work? It's close to the 600 bp ladder band & some are between 600bp

and 700bp.

July 8th, 2015

For Jeffrey and Eleanor:

1. Take yellow capped culture tubes labeled "HWP1", "ECadherin", "CB008DB" from 30'C incubation box, spinny thing
2. Pour into snap-top falcon tubes label with "HWP1", "ECadherin", "CB008DB"
3. Pellet in big centrifuge @ 3000 rpm for 5min
4. Pour out supernatant
5. Resuspend in S-Raff (5mL), put back into yellow capped culture tubes
6. Put back into 30'C incubator spinner.

PCR mFa #3

1. Add 2.5 ul of FW primer (131)
2. Add 2.5 ul of RV primer (132)
3. Add 0.5 pGem 41 DNA Template
4. Add 20 ul of Phusion Master Mix (b/c we ran out so we have 5ul less)
5. Add 19.5 ul of water

Touchdown PCR Protocol Phusion:

Initial Denature	98'C	30s	
10 cycles of			
Denature	98'C	10s	
Anneal	55'C	20s	
-1'C per cycle			
Extenstion	72'C	45s	
30 cycles of			
Denature	98'C	10s	
Anneal	55'C	20s	
Extension	72'C	45s	
Final Extension	72'C	5m	
Hold	4'C	Forever	

Miniprep Liqid Culture (#3 T64-M64) (#1 Old Digest) (#2 OD)

1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

DNA Sequencing

These are all plasmids (pNH605) with the promoter (LexAOps) and has the Apa1 cut site and an extra 10bp in it with nothing else.

1. Pipette 5 ul of plasmid into 1.5 ml tubes

```
        Tube #1: "OD #1"
        Tube #2: "OD #2"
        Tube #3: "T64-M64 #3"
PCR mFa #3 Gel
    Did a 1.5% Agarose Gel with 35 mL and 3.5 ul of Sybersafe
        Key:
            1. Ladder bled over
            2. 2 log DNA Ladder
            3. #1 T64 (Td-Tomato) Colony PCR
            4. #2 T64 (Td-Tomato) Colony PCR
            5. #3 T64 (Td-Tomato) Colony PCR
            6. #4 T64 (Td-Tomato) Colony PCR
            7. #5 T64 (Td-Tomato) Colony PCR
            8. #6 T64 (Td-Tomato) Colony PCR
            9. mFa #4 (PCR)
            10. 2 Log DNA Ladder
    Please see box for gel photo "T64 (Td -Tomato) Colony PCR + mFα #4 Edit 1"
```

July 9th, 2015

```
To do:
    1. Get new primers (Apa1 & Xho1) (Dilute)
    2. PCR LexAOps for the 6th time
    3. Digest LexAOps, Hy111E2, and Hy86E3
    4. Gel Extract
    5. Phosphotase the plsmid digest right away before ligation
Dilute Primers for Working Stock
    Forward primer (Apa1)                   10 ul of primer + 90 ul of water
    Reverse primer (Xho1)                   10 ul of primer + 90 ul of water
PCR LexAOps #6
```

| Tubes | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | |--------|--------||--------|--------||--------|--------||--------| |FW Primer |2.5 ul|2.5ul|2.5 ul|2.5 ul|2.5ul|2.5 ul|2.5 ul|2.5ul| |RV Primer|2.5 ul|2.5ul|2.5 ul|2.5 ul|2.5ul|2.5 ul|2.5 ul|2.5ul| |DNA Template (T64 Td-Tomato) |0.5 ul|0.5ul|0.5 ul|0.5 ul|0.5ul|0.5 ul|0.5 ul|0.5ul| |GoTaq Green|25ul|25 ul|25ul|25ul|25 ul|25ul|25ul|25 ul| |Water (ddH2O)|19.5ul|19.5ul|19.5ul|19.5ul|19.5ul|19.5ul|19.5ul|19.5ul| LexAOps #6 Gel 1.5% Agarose Gel with 100 mL Agarose and 10 ul of Sybersafe Key: 1. 2 Log DNA Ladder 10 ul 2. LexAOps PCR #6 (#1) 10 ul 3. LexAOps PCR #6 (#2) 5 ul 4. LexAOps PCR #6 (#3) 5 ul 5. LexAOps PCR #6 (#4) 5 ul 6. LexAOps PCR #6 (#5) 5 ul 7. LexAOps PCR #6 (#6) 5 ul 8. LexAOps PCR #6 (#7) 5 ul 9. LexAOps PCR #6 (#8) 5 ul 10. 2 Log DNA Ladder 10 ul Please see the box for the gel photo under "LexAOps PCR #6 45mins" LexAOps (8), Hy111E2, Hy86E3 Digest Promoter Digest (LexAOps) ---> Sample #1-8 Promoter Digest: 1. Add 5 ul CutSmart Buffer to your PCR tube 2. Add 0.5 Apa1 restriction enzyme to the tube. 3. Vortex to mix well. 4. Incubate @ room temperature for at least 1 hour. 5. Add 0.5 ul Xho1 restriction enzyme to the tube. 6. Vortex to mix well. 7. Incubate @ 37 degree for at least 1 hour. Error: In tube #1 for LexAOps PCR #6 we added 5 ul of Apa1 instead of 0.5 ul Plasmid Digest (2 different digest): 1. Take 10 ul of plasmid from the plasmid (pNH605) stock (Hy111E2 and Hy86E3) into a new tube. 2. Add 3 ul CutSmart Buffer and 16 ul of water to your tube. 3. Add 0.5 ul Apa1 restriction enzyme to the tube. 4. Vortex to mix well. 5. Incubate @ room temperature for @ least 1 hr. 6. Add 0.5 ul Not1 restriction enzyme to the tube. 7. Vortex to mix well. 8. Incubate @ 37 degree for @ least 1 hr.

July 10th, 2015

For Jeffrey and Eleanor:

Liquid Culture of T64-M64

1. Add 5 mL of LB + Carb into a falcon tube
2. Mix 1 colony and place into falcon tube
3. 37'C o/n on shaker

Make 1.5% Agarose

1. Pour 400 mL of 1X TAE Buffer into 500 mL flask
2. Weigh 6g of Agarose Powder
3. Mix and microwave

Make 1X TAE Buffer

1. Use 900 mL flask
2. Pour 980 mL of Mili-Q into a flask
3. Pour 20 mL of 50X TAE Buffer
4. Mix and label

Gel Extract for LexAOps Digest

1.5% Agarose Gel with 100 mL Agarose and 10 ul of Sybersafe

Key:

1. 2 Log DNA Ladder	10 ul
2. Promoter #1	30 ul
3. Promoter #1	19 ul
4. Promoter #2	30 ul
5. Promoter #2	19 ul
*6. Promoter #3	30 ul
*7. Promoter #3	20 ul
*8. Promoter #4	30 ul
*9. Promoter #4	20 ul
10. Promoter #5	30 ul
11. Promoter #5	20 ul
12. Promoter #6	30 ul
13. Promoter #6	20 ul
14. 2 Log DNA Ladder	10 ul
15. Jeffrey Sample	30 ul

Ran this gel for 100 volts for 40 mins

Please see the box for the gel photo under "LexAOps Digestion #4"

Extracted the bands from the lanes with the "*"

To do

1. Gel Extract
2. Phophotase plasmid digest
3. Do a ligation for the plasmid and promoter
4. Gel for ligation
5. Transform into E.coli
6. Liquid culture the transformation

Gel Extraction of LexAOps Digest

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the

tube.

a. #3 Gel weight: 113 mg

i. Buffer QG: 339 ul

b. #4 Gel weight: 133 mg

i. Buffer QG: 399 ul

3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol.

a. Isopropanol #3: 113 ul

b. Isopropanol #4: 133 ul

5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nanodrop

#3: 17.28 ng/ul

#4: 17.51 ng/ul

Gel Extraction for plasmids (Hy111E2 and Hy86E3) & LexAOps

Used a 1.5% Agarose Gel for gel extracting

Key:

1. 2 Log DNA Ladder	10 ul
2. Plasmid Hy86E3	36 ul
3. Plasmid Hy111E2	32 ul
4. Promoter #7	17 ul
5. Promoter #8	30 ul
6. Promoter #8	13.6 ul
7. 2 Log DNA Ladder	10 ul

Ran the gel for 100 volts at 45 mins

Please see box for the gel photos under "LexAOps + pNH605 Digestion #4"

Resuts:

We received bands but it's so big. We're going to do another digest of promoter (LexAOps)

Gel Extraction of plasmids Digest

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.

a. Hy86E3 Gel weight: 34 mg

i. Buffer QG: 100 ul

b. Hy111E2 Gel weight: 75 mg

i. Buffer QG: 230 ul

3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol.

a. Isopropanol Hy86E3: 34 ul

b. Isopropanol Hy111E2: 75 ul

5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

```
Nanodrop
    Hy86E3: 19.18 ng/ul
    Hy111E2: 71.52 ng/ul
```

July 13th, 2015

```
Plasmid Redigest (Hy111E2 and Hy86E3)
    1. Take 10 ul of plasmid from the plasmid (pNH605) stock (Hy111E2 and          Hy86E3) into a new tube.
    2. Add 3 ul CutSmart Buffer and 16 ul of water to your tube.
    3. Add 0.5 ul Apa1 restriction enzyme to the tube.
    4. Vortex to mix well.
    5. Incubate @ room temperature for @ least 1 hr.
    6. Add 0.5 ul Not1 restriction enzyme to the tube.
    7. Vortex to mix well.
    8. Incubate @ 37 degree for @ least 1 hr.
                            Total volume: 58 ul
```

July 14th, 2015

```
Plasmid Redigest
    Hy86E3                                        12 ul of loading dye
    Hy111E2                                    12 ul of loading dye
        Key:
            1. 2 Log DNA Ladder                   10 ul
            2. Hy86E3                              35 ul
            3. Hy86E3                               31 ul
            4. Hy111E2                             35 ul
            5. Hy111E2                             30 ul
                        Ran for 75 volts for 40 mins
    Gel photo in box under "Hy86E3 + Hy111E2 Re-Digest"
Gel Extraction of Plasmid Redigest:
    1. Using a clean, sharp blade, cut out a the bands desired from the gel
    2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the        volume of the gel) to the
tube.
        a. Weight: 194 mg
            i. Buffer QG: 580 ul
    3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid          should be yellow)
        a. Isopropanol: 194 ul
    4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol.
    5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
    6. Discard the flow-through
    7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
    8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
    9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and          centrifuge for 1 min.
Nanodrop
    Hy111E2: 20.84 ng/ul
Phosphotase treat plasmid
```

Do this before ligating the promoter with the Plasmid to keep it from annealing to other sites besides the desired one.

1. Add 1 ul of Phosphotase
2. Incubate @ 37'C for 1 hour
3. Heat inactivate @ 65'C for 10 min

Ligase LexAOps + Hy111E2 #6

Ligation	LexAOps Digest #3	LexAOps Digest #4	(-) Control
Ligase Buffer	2 ul	2 ul	2 ul
Vector DNA	2.6 ul	2.6 ul	2.6 ul
Inseert DNA	1 ul	1 ul	0.0 ul
ddH2O	13.4 ul	13.4 ul	13.4 ul
T4 DNA Ligase	1 ul	1 ul	1 ul

Transformation

We only received 3 colonies from LexAOps + Hy111E2 #3 (8X) and 1 colony from LexAOps + Hy111E2 #4 (8X)

July 15th, 2015

Colony PCR LexAOps #6

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later.

   Repeat for all 6 colonies (1 tube per 1 colony)
5. Make a "Master Mix" of PCR components (PCRing 5 colonies, make for 6)

	1x Reaction	7x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	60 ul
b. 10 uM FW primer (pTEFm3)	1 ul	6 ul
c. 10 uM REV primer (Bar 1 #93)	1 ul	6 ul
d. water	3 ul	18 ul
		Total: 90 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.
7. Put PCR strips in thermocycler (saved file "Touchdown Phu")

TouchDownPhusion

Initial Denaturation	98'C	30s
10 Cycles		
Denaturation	98'C	10s
Annealing	55'C	20s
(-1'C Per Cycle)		
Extension	72'C	30s
30 Cycles		
Denaturation	98'C	10s
Annealing	55'C	20s
Extension	72'C	30s
Final Extension	72'C	5m
Hold	4'C	Forever

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel

a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul

9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Colony PCR Gel

1.5% of Agarose Gel (100 ml + 10 ul of Sybersafe)

Ran @ 90'C for 55 mins

Key:

1. Ladder 10 ul
2. Colony #1 10 ul
3. Colony #2 10 ul
4. Colony #3 10 ul
5. Colony #4 10 ul
6. Empty
7. (-) Control 10 ul
8. Ladder 10 ul

Please see the box for the gel photo under "LexAOps #6 Colony PCR"

LexAOps #6 Liquid Culture

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add our colony to PCR tubes into falcon tubes
3. Vortex and 4'C fridge o/n
4. Place in 37'C incubator o/n

Reconstituted Primers for Gibson Assembly (LexAOps)

Gibson Uses overhangs instead of cut sites

1. LexAOps (+GFP) REV #147
   27.5 nmole x 10 = 275 ul
2. LexAOps (+mFa) REV #148
   27.8 nmole x 10 = 278 ul
3. LexAOps (+Ste2) REV #149
   27.2 nmole x 10 = 272 ul
4. LexAOps (+pNH605) FW #150
   28.4 nmole x 10 = 284 ul

Dilution

10 ul of primer + 90 ul of water for working stock

PCR 3 Reactions

1. LexAOps (+ GFP)
   a. 2.5 ul of FW Primer: 150 (+ pNH605)
   b. 2.5 ul of RV Primer: 147 (+ GFP)
   c. 0.5 ul DNA Template T-64 (td Tomoato)
   d. 25 ul of GoTaq Green Master Mix
   e. 19.5 ul of Water (ddH2O)
2. LexAOps (+ mFa)
   a. 2.5 ul of FW Primer: 150 (+ pNH605)
   b. 2.5 ul of RV Primer: 148 (+ mFa)
   c. 0.5 ul DNA Template T-64 (td Tomoato)

```
            d. 25 ul of GoTaq Green Master Mix
            e. 19.5 ul of Water (ddH2O)
        3. LexAOps (+ Ste2)
            a. 2.5 ul of FW Primer: 150 (+ pNH605)
            b. 2.5 ul of RV Primer: 149 (+ Ste2)
            c. 0.5 ul DNA Template T-64 (td Tomoato)
            d. 25 ul of GoTaq Green Master Mix
            e. 19.5 ul of Water (ddH2O)
                Touchdown PCR Protocol Phusion:
                            Volume = 50 ul and liquid temp. = 105'C
                    Initial Denature                98'C                30s
                    10 cycles of
                        Denature                    98'C                10s
                        Anneal                  55'C                20s
                            -1'C per cycle
                        Extenstion          72'C                30s
                     30 cycles of
                           Denature                    98'C                10s
                           Anneal                  55'C                20s
                           Extension               72'C                30s
                    Final Extension               72'C              5m
                     Hold                             4'C               Forever
mFa PCR #4 Using Gibson Assembly
    1. 2.5 ul FW #131
    2. 2.5 ul REV #132
    3. 0.5 ul pGEM 41
    4. 25 ul Phusion Master Mix
    5. 19.5 ul Water
        Gradiant PCR Protocol
            Initial Denature                    98'C                30s
                35 Cycles
                    Denature                    98'C                10s
                    Annealing                  55'C - 65'C      20s
                    Extension                  72'C            45s
            Final Extension                  72'C           5 min
            Hold                                4'C             Forever
Liquid Culture of LexAOps + Hy111E2 #4
Under a flame:
        1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
        2. Add 20 ul of our colony to PCR tubes into falcon tubes
        3. Vortex and 4'C fridge o/n
        4. Place in 37'C incubator o/n
```

July 16th, 2015

```
Made 1X TAE Buffer
    Add 980 ml of Milli-Q water
    20 ml of 50X TAE Buffer
```

Gel for LexAOps & mFa #4 Gibson

1.5% Agarose Gel

This was for 8 mFa samples and 3 LexAOps Gibson Assembly

Key:

1. 2 Log DNA Ladder	10 ul
2. LexAOps (+ GFP)	6 ul
3. LexAOps (+ mFa)	6 ul
4. LexAOps (+ Ste2)	6 ul
5. Empty	
6. mFa #1	6 ul
7. mFa #2	6 ul
8. mFa #3	3.2 ul
9. mFa #4	6 ul
10. mFa #5	6 ul
11. mFa #6	6 ul
12. mFa #7	6 ul
13. Empty	
14. mFa #8	6 ul
15. Empty	
16. Ladder	10 ul

Please see the box for gel photo "LexAOps Gibson + mFa #4 PCR 40mins"

Results:

1. Bands are a bit faint for LexAOps
2. LexAOps (+ Ste2) band is super faint
    - Re -PCR using old LexAOps as DNA Template
3. mFa bands #1-3 have the brightest bands @ 500 bp
    - Reran on 4% Agarose Gel and extracted

Miniprep Liquid Culture of LexAOps + Hy111E2 #4

1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

Nanodrop

LexAOps #1	495.4 ng/ul
LexAOps #2	811.3 ng/ul
LexAOps #3	770.3 ng/ul
LexAOps #4	690.2 ng/ul

DNA Sequencing

1. Pipette 5 ul of plasmid into 1.5 ml tubes
    Tube #1: "1" No promoter or pTET so digest worked but not ligation

```
        Tube #2: "2"    Failed
        Tube #3: "3"    No promoter or pTET so digest worked but not ligation
        Tube #4: "4"    No promoter or pTET so digest worked but not ligation
Made 4% Agarose Gel
    1. 100 ml flask
    2. 50 ml of 1x TAE Buffer
    3. 2g of Agarose Powder
mFa PCR Gel
    Used the 4% Agarose Gel
    Ran @ 100 volts for 45 mins then an extra 30 mins for 120 mins
        Key:
            1. Ladder                                   10 ul
            2. mFa #1                                   27 ul
            3. mFa #1                                   14 ul
            4. mFa #2                                   20 ul
            5. mFa #2                                   20 ul
            6. mFa #3                                   20 ul
            7. mFa #3                                   20 ul
            8. Ladder                                   10 ul
        Please see box for gel photo "mFa PCR #4 Gel #2 75 mins"
LexAOps (+ Ste2) PCR #2
    LexAOps (promoter) with Ste2 overhangs for Gibson
        1. 10 uM FW Primer #150 (+ pNH605)                  2.5 ul
        2. 10 uM REV Primer #149 (+ Ste2)                    2.5 ul
        3. LexAOps (Old Digest #3)                          0.5 ul
        4. 2x Phusion Master Mix                              25 ul
        5. Water                                            19.5 ul
                                                          Total: 50 ul
    Touchdown PCR Protocol
        Initial Denature                        98'C            30s
                10 cycles of
                    Denature                    98'C            10s
                    Anneal                  55'C            20s
                        -1'C per cycle
                    Extenstion              72'C            30s
                30 cycles of
                    Denature                    98'C            10s
                    Anneal                  55'C            20s
                    Extension               72'C            30s
                Final Extension             72'C            5m
                Hold                            4'C             Forever
```

July 17th, 2015

```
LexAOps (+ Ste2) PCR #2 gel
    35 ml of 1.5% Agarose Gel + 3.5 ul Sybersafe
                Ran  @ 100 volts for 30 miles
        Key:
```

#1-4 Lanes Blank

5. Ladder 10 ul

6. Blank

7. LexAOps (+ Ste2) PCR 6 ul

Please see box for gel photo "LexAOps (+Ste2) PCR #2"

Gel Extract mFa DNA

Extracted lanes 4-7

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.

a. Gel Weight: 242 mg

i. Buffer QG: 726 ul + 726 ul +

3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol
5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: + 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nanodrop

mFa: 42.48 ng/ul

Plasmid Digest pNH605 (Hy86E3)

1. 3 ul of CutSmart Buffer to PCR tube
2. 16 ul of Water
3. 0.5 ul of Apa1 enzyme
4. Vortex
5. Incubate @ room temperture for 1 hour
6. Add 0.5 ul of Not1 enzyme
7. Vortex
8. Incubate @ 37'C for 1 hour

Gel Loading:

1% Agarose Gel	35mL
SyberSafe	3.5uL

Key:

2. 2-Log DNA Ladder	10.0uL
3. Empty	0.0uL
4. Hy86E3 Digestion	36.0uL

Please see box for gel photo "Hy86E3 Gibson Digest"

Gel Results:

-Good plasmid band

-Excised pTET and GFP band is visible

-Expected around 7kb size bands

Gel Extract pNH605 (Hy86E3) plasmid

1. Using a clean, sharp blade, cut out a the bands desired from the gel
2. Weigh the gel slice in a colorless tube. Add Buffer QG (3 times the volume of the gel) to the tube.

Weight: 193 mg

a. Buffer QG: 579 ul

3. Incubate @ 50 degree for 10 mins (gel should be dissolved & liquid should be yellow)
4. Add 1 gel volume (gel weight in ul form 1 mg = 1 ul) of isopropanol.
Isopropanol: 193 ul
5. Dump the 1.5 ml tube's content into a spin column & centrifuge for 1min
6. Discard the flow-through
7. To wash: add 0.75 ml of Buffer PE to column and centrifuge for 1 min
8. Discard flow-through & centrifuge the column for 1 min @ 13,000rpm
9. Elute DNA: Add 50 ul of water, let the column stand for 1 min, and centrifuge for 1 min.

Nanodrop

Hy86E3 Gibson: 47.32 ng/ul (for gel extraction)

L Blank:	327.1 ng/ul
L mFa:	487 ng/ul
L Ste2:	450.5 ng/ul
Ste2 (+ LexAOps):	396 ng/ul

The concentration for these pieces were too high so we needed to do a PCR Purification.

PCR Purification

Samples need to purify

1. L Blank (LexAOps + GFP)
2. L mFa
3. L Ste2
4. Ste2 (LexAOps)

Protocol

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix
    a. L GFP: 750 ul*
    b. L mFa: 450 ul
    c. L Ste2: 750 ul*
    d. Ste2 (+ LexAOps): 750 ul*

*Error: Added too much Buffer PB

2. Check mixture that it is a yellow color
3. Pour supernatant into a spin column and centrifuge for 1 min to bind the DNA
4. Discard the flow through
5. To wash, add 0.75 ml of Buffer PE to the column and centrifuge for 1 min
6. Discard flow through & dry spin
7. Place the column in a 1.5 ml microcentrifuge tube
8. To elute DNA, add 50 ul of water to the center of the column
9. Let it stand 1 min then centrifuge it for 1 min

Nanodrop after PCR Purification

LexAOps (+ GFP)	30.62 ng/ul
LexAOps (+ mFa)	49.27 ng/ul
LexAOps (+ Ste2)	114.8 ng/ul
Ste2 (+ LexAOps)	101.5 ng/ul

Gibson Assembly:

Use half of the recommended reagents (a 1:1 ratio of Gibson Mix & DNA)

1. Old Plasmid w/GFP w/ LexAOps + Apa1 + Xho1 Digest
2. New Digest Plasmid
    a. LexAOps w/ mFa + plasmid
    b. LexAOps w/ Ste2 + plasmid
3. Negative Control (no insert DNA)

a. Old Digest
b. New Digest

4. Positive Control Plate
-5 ul of Mix
-5 ul of Gibson

Protocol:

1. LexAOps + mFa + Hy86E3
a. Vector DNA (50ng): 1.06uL
b. LexAOps DNA (15ng): 0.30uL
c. mFa DNA (10ng): 0.24uL
d. 2x Gibson Master Mix: 5.00uL
e. Water: 3.40uL
Total: 10.0uL

2. LexAOps + Ste2 + Hy86E3
a. Vector DNA (50ng): 1.06uL
b. LexAOps DNA (15ng): 0.13uL
c. Ste2 DNA (10ng): 0.30uL
d. 2x Gibson Master Mix: 5.00uL
e. Water: 3.51uL
Total: 10.0uL

3. LexAOps + GFP + Hy111E2
a. Vector DNA (50ng): 2.40uL
b. LexAOps DNA (15ng): 0.50uL
c. 2x Gibson Master Mix: 5.00uL
d. Water: 2.10uL
Total: 10.0uL

4. (-) Control
a. Vector DNA (50ng): 1.10uL
b. 2x Gibson Master Mix: 5.00uL
c. Water: 3.90uL
Total: 10.0uL

5. (+) Control
a. Positive Control Mix: 5.00uL
b. 1x Gibson Master Mix: 5.00uL
Total: 10.0uL

Incubate the samples @ 50'C for 60 mins, then hold @ 4'C

LexAOps + (mFa/Ste2/GFP) + pNH605 Transformation

a. LexAOps + mFa + Hy86E3
b. LexAOps + Ste2 + Hy86E3
c. LexAOps + GFP + Hy111E2
d. (-) Control
e. (+) Control

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 2uL of Gibsoned LexAOps + mFa + Hy86E3 to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes

6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over weekend at room temperature

July 20th, 2015

Colony PCR

1. LexAOps + mFa + Hy86E3 (1X)
    a. FW Primer: #150
    b. REV Primer: #132
2. LexAOps + Ste2 + Hy86E3(1X)
    a. FW Primer: #150
    b. REV Primer: #134
3. LexAOps + GFP + Hy111E2
    a. FW Primer: #150
    b. REV Primer: #142

Protocol

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later. Repeat for all 6 colonies (1 tube per 1 colony)
5. Make a "Master Mix" of PCR components (PCRing 6 colonies, make for 7)

	1x Reaction	7x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	70 ul
b. 10 uM FW primer	1 ul	7 ul
c. 10 uM REV primer	1 ul	7 ul
d. water	3 ul	21 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.
7. Put PCR strips in thermocycler for the cycle (saved file "GoTaq)

	95	5 min
30x:	95	45s
	55	30s
	72	1' per kb
	72	10'

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel
    a. While loading gel #3, we didn't have 10 ul so we loaded 9ul
9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate

them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Colony PCR Gel

1.5% Agarose, 100 ml of Agarose with 10 ul of Sybersafe

Key:

1. Ladder	10 ul
2. LexAOps + mFa #1	5 ul
3. LexAOps + mFa #2	5 ul
4. LexAOps + mFa #3	5 ul
5. LexAOps + mFa #4	5 ul
6. LexAOps + mFa #5	5 ul
7. LexAOps + mFa #6	5 ul
8. LexAOps + Ste2 #1	5 ul
9. LexAOps + Ste2 #2	5 ul
10. LexAOps + Ste2 #3	5 ul
11. LexAOps + Ste2 #4	5 ul
12. LexAOps + Ste2 #5	5 ul
13. LexAOps + Ste2 #6	5 ul
14. LexAOps + (GFP) #1	5 ul
15. LexAOps + (GFP) #2	5 ul
16. LexAOps + (GFP) #3	5 ul
17. LexAOps + (GFP) #4	5 ul
18. LexAOps + (GFP) #5	5 ul
19. LexAOps + (GFP) #6	5 ul
20. Ladder	10 ul

Ran @ 100 volts for 30 mins

Please see Box for gel photo "LexAOps + mFa,Ste2,GFP Colony PCR"

Results:

-Several bands for LexAOps + mFa

-Lane 7 Appeared to have the correct size band

-LexAOps + Ste2 bands too small

-No bands for LexAOps (+GFP)

Colony PCR LexAOps + Ste2 & LexAOps + GFP #2

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later.

Repeat for all 6 colonies (1 tube per 1 colony)

5. Make a "Master Mix" of PCR components (PCRing 6 colonies, make for 7)

	1x Reaction	7x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	70 ul
b. 10 uM FW primer (pTEFm3)	1 ul	7 ul
c. 10 uM REV primer (Bar 1 #93)	1 ul	7 ul
d. water	3 ul	21 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.
7. Put PCR strips in thermocycler for the cycle (saved file "GoTaq)

	95		5 min
30x:	95		45s
	55		30s
	72		1' per kb
72			10'

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel
    a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul
9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Colony PCR LexAOps + Ste2/GFP + Hy86E3/Hy111E2 #2

1. Filled 16 PCR tubes with 25uL of water
2. Created PCR Master Mixes:
    a. LexAOps + Ste2:

i. 2x GoTaq Green PCR Master Mix	90.0uL
ii. 10uM FW Primer-150 [LexAOps (+pNH605)]	9.0uL
iii. 10uM RV Primer-134 [Ste2 (+pNH605)]	9.0uL
iv. Water	27.0uL

    b. LexAOps (+GFP):

i. 2x GoTaq Green PCR Master Mix	90.0uL
ii. 10uM FW Primer-150 [LexAOps (+pNH605)]	9.0uL
iii. 10uM RV Primer-147 [LexAOps (+GFP)]	9.0uL
iv. Water	27.0uL

3. Transferred Colonies:
4. Transferred 1 colony to 8 PCR tubes from 1x LexAOps+Ste2+Hy86E3 experiment plate and mixed by pipetting up and down
5. Transferred 1 colony to 8 PCR tubes from 1x LexAOps+GFP+Hy111E2 experiment plate and mixed by pipetting up and down
    a. Transferred 5uL from each PCR tube to its associated clean tube
    b. Vortexed and centrifuged

Thermocycler Protocol:

Initial Denaturation		95'C	5m
30 Cycles			
	Denaturation	95'C	45s
	Annealing	55'C	30s
	Extension	72'C	2m
Final Extension		72'C	10m
Hold		4'C	Forever

Liquid Culture for LexAOps + mFa + Hy86E3

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add 20 ul of LexAOps + mFa #6 from colony PCR into falcon tubes
3. Vortex and 4'C fridge o/n
4. Place in 37'C incubator o/n

July 21st, 2015

Colony PCR LexAOps + Ste2 & LexAOps + GFP Gel

1% Agarose Gel 100 ml

SyberSafe 10.0 ul

Key:

1. 2-Log DNA Ladder 10.0 ul
2. LexAOps + Ste2 #1 5.0 ul
3. LexAOps + Ste2 #2 5.0 ul
4. LexAOps + Ste2 #3 5.0 ul
5. LexAOps + Ste2 #4 5.0 ul
6. LexAOps + Ste2 #5 5.0 ul
7. LexAOps + Ste2 #6 5.0 ul
8. LexAOps + Ste2 #7 5.0 ul
9. LexAOps + Ste2 #8 5.0 ul
10. 2-Log DNA Ladder 10.0 ul
11. LexAOps (+GFP) #1 5.0 ul
12. LexAOps (+GFP) #2 5.0 ul
13. LexAOps (+GFP) #3 5.0 ul
14. LexAOps (+GFP) #4 5.0 ul
15. LexAOps (+GFP) #5 5.0 ul
16. LexAOps (+GFP) #6 5.0 ul
17. LexAOps (+GFP) #7 5.0 ul
18. LexAOps (+GFP) #8 5.0 ul
19. 2-Log DNA Ladder 10.0 ul

Ran at 100 volts for 30 mins

Gel Results:

-See Gel Folder for gel labeled "LexAOps + Ste2,GFP PCR Colony #2"

-Several bands for Ste2

-Lanes 4,7, and 9 appear to be the correct size

-Only one band for LexAOps (+GFP) which appears correct

LexAOps + Ste2/GFP + Hy86E3/Hy111E2 Liquid Culture

1. Labeled 4 culturing tubes
   a. L Ste2 #3
   b. L Ste2 #6
   c. L Ste2 #8
   d. L GFP #6
2. Turned on bunson burner
3. Added 5mL of LB+Carb media to tubes
4. Transfered 20uL of cell solution from each correct colony PCR [(LexAOps + Ste2:#3,6,8) (LexAOps (+GFP):#6)] to culturing tubes
5. Incubated overnight

LexAOps + mFa + Hy86E3 Miniprep

LexAOps + mFa + Hy86E3 Miniprep:

1. Centrifuged liquid cultures for 10 mins
2. Resuspended cells in 250uL Buffer P1
3. Added 250uL Buffer P2. Mixed by inverting 6 times
4. Added 350uL Buffer N3. Mixed by inverting 6 times
5. Centrifuged for 10 minutes

```
        6. Poured supernatant into a QIAprep spin column
        7. Centrifuged for 30 seconds. Discarded flow-through
        8. Added 0.75mL Buffer PE to column to wash. Centrifuged for 30 second
        9. Discarded flow-through. Centrifuged empty for 1 minute
        10. Placed column in a new 1.5mL tube. Added 50uL water to column. Let          stand for 1 minute
        11. Centrifuged for 1 minute.
Nanodrop:
    LexAOps + mFa + Hy86E3                          645.2ng/uL
DNA Sequencing:
    Sent 5uL to Quintara for DNA sequencing & received
```

pGEM 47 (LexAOps + mFα + Hy86E3)

July 22nd, 2015

```
Miniprep LexAOps + Ste2 + Hy86E3 and LexAOps + GFP + Hy111E2
    1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
    2. Add 250 ul Buffer P1 (found in refridgerator)
    3. Transfer to a microcentrifuge tube
    4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
    5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
    6. Centrifuge for 30s. Discard the flow-through
    7. Apply the supernatants from step 6 to the spin column by pipetting
    8. Centrifuge for 30s. Discard the flow-through
    9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for        30s
    10. Discard flow-through & centrifuge @ full speed for 1 min to remove         residual wash buffer
    11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA,       add 50 ul of water to the
center of the spin column, let it stand for       1 min, & centrifuge for 1 min.
Nanodrop
    1. LexAOps + Ste2 #3                                    685.4 ng/ul
    2. LexAOps + Ste2 #6                                    667.8 ng/ul
    3. LexAOps + Ste2 #8                                    480.4 ng/ul
    4. LexAOps + GFP #6                                  596.5 ng/ul
DNA Sequencing
    1. Pipette 5 ul of plasmid into 1.5 ml tubes
        Tube #1: "3"
        Tube #2: "6"
        Tube #3: "8"
        Tube #4: "GFP"
                Received:
```

pGEM 49 (LexAOps + Ste2 + Hy86E3)

pGEM 50 (LexAOps + GFP + Hy111E2)

```
Combining pGEM 48 + terminator + pGEM 49
    Digest pGEM 47
        1. 5 ul of pGEM 47
        2. 2 ul CutSmart Buffer
        3. 12.5 ul Water
        4. 0.5 ul Not1
        5. Vortex
        6. Incubate @ 37'C for 4 hours
    PCR Purification
        Get rid of the buffers and enzymes to have the end product of only DNA
        Samples need to purify
            1. Digested pGEM 47 (LexAOps + mFα)
        Protocol
            1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample & mix
                a. pGEM 47: 100 ul
        2. Check mixture that it is a yellow color
        3. Pour supernatant into a spin column and centrifuge for 1 min to            bind the DNA
        4. Discard the flow through
        5. To wash, add 0.75 ml of Buffer PE to the column and centrifuge for          1 min
        6. Discard flow through & dry spin
        7. Place the column in a 1.5 ml microcentrifuge tube
        8. To elute DNA, add 50 ul of water to the center of the column
        9. Let it stand 1 min then centrifuge it for 1 min
    Nanodrop after PCR Purification
        pGEM 47                                 97.84 ng/ul
```

July 23rd, 2015

```
Reconstitute the Primers
    LexAOps (+Apa1/tCyc1) FW 155
            31.5 nmoles x 10 = 315 ul
    Ste2 (+ Sbf1/tAdh1) REV 156
            36 nmoles x 10 = 360 ul
    LexAOps (+ Apa1/tENO2) FW 157
            30.9 nmoles x 10 = 309 ul
Dilute Primers
    In a microcentrifuge tube, add 90 ul of water and 10 ul of each primer
PCR LexAOps (+ ApaI/tCyc1) + Ste2 (+ Sbf1/tAdh1) with Overhangs
    1. Forward Primer 155 (+ApaI/tCyc1)                          2.5 ul
    2. Reverse Primer 156 (+Sbf1/tAdh1)                        2.5 ul
    3. Water (ddH2O)                                             19.5 ul
    4. Template DNA (pGEM 49)                                     0.5 ul
    5. 2X Phusion Master Mix                                      25 ul
                                                          Total: 50 ul
            Thermocycler Protocol:
                Initial Denaturation            98'C                30s
                35 Cycles
```

```
                    -Denaturation                98'C            10s
                    -Annealing                   55'C             20s
                    -Extension                   72'C            30s
             Final Extension                  72'C              5m
             Hold                               4'C            Forever
```

July 24th, 2015

```
PCR LexAOps (+ ApaI/tCyc1) + Ste2 (+ Sbf1/tAdh1) with Overhangs Gel
    -1% Agarose Gel             35uL
    -SyberSafe                  3.5uL
        Key:
            1. Blank
            2. 2-Log DNA Ladder              10.0 ul
            3. LexAOps + Ste2                   6.0 ul
                        Ran at 100 volts for 30 mins
        Gel Results:
            -See Gel Folder for gel labeled "LexAOps + Ste2 (OH)"
            -Band appeared to be slightly smaller than expected
PCR Purification
        1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix
               a. L Ste2 OH: 45 ul
        2. Check mixture that it is a yellow color
        3. Pour supernatant into a spin column and centrifuge for 1 min to            bind the DNA
        4. Discard the flow through
        5. To wash, add 0.75 ml of Buffer PE to the column and centrifuge for          1 min
        6. Discard flow through & dry spin
        7. Place the column in a 1.5 ml microcentrifuge tube
        8. To elute DNA, add 50 ul of water to the center of the column
        9. Let it stand 1 min then centrifuge it for 1 min
    Nano-Drop:
        -Tested 1.5uL of each sample
            LexAOps + Ste2:             104.9ng/uL
Gibson Assembly
    LexAOps (+ApaI/tCyc1) + Ste2 (+Sbf1/tAdh1) + tCyc1 (+mFa) + pGEM47
        Protocol:
            1. LexAOps + mFa + tCyc1 + Ste2
                a. Vector DNA [pGEM47] (50ng)              0.55uL
                b. tCyc1 DNA (15ng)                      0.10uL
                c. LexAOps + Ste2 DNA (10ng)               0.35uL
                d. 2x Gibson Master Mix                  5.00uL
                e. Water                                   4.00uL
                                              Total:      10.0uL
            2. (-) Control
                a. Vector DNA [pGEM47] (50ng)              0.55uL
                b. 2x Gibson Master Mix                  5.00uL
                c. Water                                   4.45uL
                                              Total:      10.0uL
```

3. (+) Control
a. Positive Control Mix 5.00uL
b. 2x Gibson Master Mix 5.00uL
Total: 10.0uL
4. Incubation:
-Incubated in thermocycler at 50'C for 60 mins
-Hold at 4'C forever

Transformation

LexAOps (+ Apa1/tCyc1) + Ste2 (+Sbf1/Adn1) + tCyc1 (+mFa) + pGEM 47

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 2uL of Gibsoned LexAOps (+ApaI/tCyc1) + Ste2 (+Sbf1/tAdh1) + tCyc1 (+mFa) + pGEM47 to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over weekend at room temperature

(-) Control Transformation:

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 2uL of Gibsoned (-) Control to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over weekend at room temperature

(+) Control Transformation

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 2uL of Gibsoned (+) Control to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes

4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Turned on Bunson Burner
7. Added 950uL of SOC into mixture
8. Incubated at 37'C for 60 minutes. Shaken at 250rpm
9. Warmed two plates (LB + Carb) to 37'C
10. Mixed cells by flicking
11. Spread 100uL cells onto one plate
12. Centrifuged remaining mixture to concentrate cells
13. Discarded excess liquid and vortexed resuspended cells
14. Spread 100uL (8x) cells onto second plate
15. Incubated both plates over weekend at room temperature

July 27th, 2015

Colony PCR (LexAOps + mFa) + (tCyc1) + (LexAOps + Ste2) Transformation

1. Pick 4-6 colonies to test if they have our DNA & get 2 strips of 6 PCR tubes.
2. Using the 1st strip of 6 PCR tubes, add 25 ul of sterile water.
3. Pick 1 colony using a p20 pipette tip, scoop GENTLY to get that colony. Set pipette to 5 ul and pipette up & down to mix in 25 ul of water.
4. Transfer 5 ul of this into your empty PCR tubes. Save remaining 20 ul for later.

Repeat for all 6 colonies (1 tube per 1 colony)

5. Make a "Master Mix" of PCR components (PCRing 8 colonies, make for 9)

	1x Reaction	9x Reaction Master
a. 2x GoTaq Green PCR Mix	10 ul	90 ul
b. 10 uM FW primer (mFa +LexAOps OH)	1 ul	9 ul
c. 10 uM REV primer (Ste2 +Sbf1/Adn1 OH)	1 ul	9 ul
d. water	3 ul	27 ul

6. Tubes containing 5 ul of bacterial cells, add 15 ul of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.
7. Put PCR strips in thermocycler for the cycle (saved file "GoTaq)

	95	5 min
30x:	95	45s
	55	30s
	72	1' per kb
	72	10'

8. Analyze products on a 1% agarose gel. GoTaq mix already has loading dye in it, so just directly load 5-10 ul of your PCR reaction into gel
    a. While loading gel #3, we didn't have 10 ul so we loaded 9 ul
9. For all positive bands on the gel, take the rest of the bacterial cells from step 1 & inoculate them into an o/n LB (+antibiotic) for miniprep.

FOR (-) CONTROL: DON'T PUT IN INSERT

Thermocycler:

Initial Denature	95 degree	5'
30 cycles of		
Denature	95 degree	42s
Anneal	55 degree	30s
Extension	72 degree	3hr 30'

Final Extension	72 degree	10'	
Hold	4 degree	forever	

Colony PCR (LexAOps + mFa) + (tCyc1) + (LexAOps + Ste2) -> Lmts Gel

1% Agarose Gel for 100 volts for 30 mins

Key:

1. 2 Log DNA Ladder
2. Colony PCR #1 LexAOps + mFa + tCyc1 + LexAOps + Ste2
3. Colony PCR #2 LexAOps + mFa + tCyc1 + LexAOps + Ste2
4. Colony PCR #3 LexAOps + mFa + tCyc1 + LexAOps + Ste2
5. Colony PCR #4 LexAOps + mFa + tCyc1 + LexAOps + Ste2
6. Colony PCR #5 LexAOps + mFa + tCyc1 + LexAOps + Ste2
7. Colony PCR #6 LexAOps + mFa + tCyc1 + LexAOps + Ste2
8. Blank
9. (-) Control #1
10. (-) Control #2
11. 2 Log DNA Ladder
12. Jeffrey Sample
13. Jeffrey Sample
14. Jeffrey Sample
15. Jeffrey Sample
16. Jeffrey Sample
17. Jeffrey Sample
18. Jeffrey Sample
19. Jeffrey Sample
20. 2 Log DNA Ladder

Please see box for gel photo under "LmtS Colony PCR"

-Received only 1 correct band in lane #4

Liquid Culture for Lmts

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add 20 ul of our colony #3 PCR tubes into falcon tubes
3. Vortex and 4'C fridge o/n
4. Place in 37'C incubator o/n

pGEM47, 49, and 50 Transformations

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into 3 1.5mL tubes. Placed on ice
2. Added 0.5uL of plasmid pGEM47, 49, and 50 to each tube. Flicked tubes 5 times to mix
3. Placed mixture on ice for 10 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Added 450uL of SOC into tubes
7. Incubated at 37'C for 60 minutes. Shaken at 250rpm
8. Warmed three plates (LB + Carb) to 37'C
9. Spread 100uL cells onto each plate
10. Incubated plates overnight at 37'C

Yeast Transformation (Part 1)

1. Added 5mL YPD to two glass culturing tubes
2. Using a toothpick, selected a sample of yeast from streak plates CB008DB and yGEM
3. Mixed cells into tubes

4. Placed in 30'C and spun overnight

July 28th, 2015

Miniprep Lmts
1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

DNA Sequencing
Pipette 5 ul of plasmid into 1.5 ml tubes into 3 separate tubes
1. a. 4 ul of DNA
   b. 2.5 ul of #136 working dilution primer
   c. 8.5 ul of H2O
2. a. 4 ul of DNA
   b. 2.5 ul of #137 working dilution primer
   c. 8.5 ul of H2O
3. a. 2.5 ul
   b. 2.5 ul H20

pGEM 53 (LexAOps + mFa + tCyc1 + LexAOps + Ste2)

Streak Plates of pGEM 47, pGEM 49, pGEM 50
The day before we transformed pGEM 47, pGEM 49, pGEM 50 into E. coli to make more for stock, however, we received a ton of colonies so we had to streak it onto a new plate to make more spread out.
1. Using a toothpick, gather a line of colonies
2. Spread onto a new warmed LB+Carb plate into a small area
3. Using a new toothpick, spread across plate with one line and then pie-wedges
4. Using a third toothpick, spread across plate again with one line and then pie-wedges
5. Incubated overnight at 37'C

Digested pGEM 50 into CB008DB
1. Need 2,000ng of pGEM 50 so about 3.4 ul
2. 0.5 ul of pME1
3. 14.1 ul of water
4. Vortex
5. Incubate @ 37'C for 2 hours

Yeast Transformations (Part 2) of pGEM 50
1. Transfer mixture from O/N Culture Dilution to new culturing tubes

```
    2. Centrifuge at 3000 for 4 mins
    3. Discard Supernatent
    4. Resuspend cells with 1mL 0.1M LiOAc
    5. Transfer over to 1.5mL tubes
    6. Centrifuge cells at 3000 for 4 mins
    7. Discard Supernatent
    8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture
        -If Necessary, split into 100uL tubes for each transformation
    9. Add 100ug (10uL) ssDNA to each 100uL sample
    10. Add 5uL of target DNA
        -1:
            -pGEM50
        -2:
            -pGEM50
            -pGEM48
    11. Add in order:
        a. 480uL 50% PEG 3350
        b. 60uL 10X TE
        c. 60uL 1M LiOAc
        d. 75uL DMSO
    12. Vortex
    13. Incubate ar 42'C for 20 mins
    14. Warm Selection Plates
        -1:
            -LEU2
        -2:
            -TRP + LEU2
    15. Centrifuge tubes at 6000rpm for 2 mins
    16. Discard Supernatent by pipetting
    17. Resuspend in 500uL YPD
    18. Centrifuge tubes at 6000rpm for 2 mins
    19. Discard most of the Supernatent, leaving ~ 50uL
    20. Resuspend cells in residual YPD
    21. Plate on selective media
    22. Incubate for 1-3 days at 30'C
```

July 29th, 2015

```
Liquid Culture of streaked plates
    1. pGEM 47
    2. pGEM 49
    3. pGEM 50
        Under a flame:
            1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
            2. Add 20 ul of our colony to PCR tubes into falcon tubes
            3. Vortex and 4'C fridge o/n
            4. Place in 37'C incubator o/n
Transform pGEM 53
```

1. Add 0.5 ul of plasmid to 25 ul of competent cells
    -make sure the competent cells are thawed (10min on ice)
    -mix gently
2. Flick tube 5 times to mix the cells & DNA
3. Place on ice for 10 min
4. Heat shock for 30s @ 42'C
5. Place on ice for 5 min
6. Add 50 ul of SOC media under fire
7. Place @ 37'C for 1 hr while shaking
8. Warm LB-Carb plates
9. Plate 100 ul of cells
10. Place plates in 37'C incubater for o/n

July 30th, 2015

Liquid Culture
    Under a flame:
        1. Add 5 mL of LB + Carb media into 5 falcon tubes (1 colonie)
        2. Add 20 ul of our colony to PCR tubes into falcon tubes
        3. Place in 37'C incubator o/n
Miniprep pGEM 47, pGEM 49, pGEM 50
    1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
    2. Add 250 ul Buffer P1 (found in refridgerator)
    3. Transfer to a microcentrifuge tube
    4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
    5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
    6. Centrifuge for 30s. Discard the flow-through
    7. Apply the supernatants from step 6 to the spin column by pipetting
    8. Centrifuge for 30s. Discard the flow-through
    9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
    10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
    11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.
Nanodrop
    pGEM 47 594.1 ng/ul
    pGEM 49 388.9 ng/ul
    pGEM 50 597.8 ng/ul
Miniprep For Jeffrey
    Samples:
        -Hwp1 (Sbf1) #1
        -Hwp1 (Sbf1) #4
        -Mgfp5 (NotI) #1
        -Mgfp5 (NotI) #5
        -Mgfp5 (Sbf1) #1
        -Mgfp5 (Sbf1) #5
    1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
    2. Add 250 ul Buffer P1 (found in refridgerator)
    3. Transfer to a microcentrifuge tube

4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

Nanodrop

- -Hwp1 (Sbf1) #1 311.9ng/uL
- -Hwp1 (Sbf1) #4 386.8ng/uL
- -Mgfp5 (NotI) #1 772.4ng/uL
- -Mgfp5 (NotI) #5 689.7ng/uL
- -Mgfp5 (Sbf1) #1 566.9ng/uL
- -Mgfp5 (Sbf1) #5 551.6ng/uL

July 31st, 2015

Miniprep pGEM 53

1. Resuspend pelleted bacterial cells (centrifuge for 10 min)
2. Add 250 ul Buffer P1 (found in refridgerator)
3. Transfer to a microcentrifuge tube
4. Add 250 ul Buffer P2 and mix throughly by inverting 6 times
5. Add 350 ul Buffer N3 & mix immediately by inverting 6 times
6. Centrifuge for 30s. Discard the flow-through
7. Apply the supernatants from step 6 to the spin column by pipetting
8. Centrifuge for 30s. Discard the flow-through
9. Wash spin column by adding 0.75ml (750 ul) Buffer PE & centrifuge for 30s
10. Discard flow-through & centrifuge @ full speed for 1 min to remove residual wash buffer
11. Place the column in a clean 1.5 ml microcentrifuge tube to elute DNA, add 50 ul of water to the center of the spin column, let it stand for 1 min, & centrifuge for 1 min.

Nanodrop

- pGEM 53 246.3 ng/ul

Yeast Transformation

- Dilute CB008DB and yGEM 127 O/N culture
  - We want our culture to be in exponential phase so we do a 1:20 dilution in media
    1. Add 9.5 ml of YPD
    2. Add 500 ul of O/N culture
    3. Incubate @ 30'C on the spinner for 3 hours
- pGEM 50 Digestion:
  1. Added 2000ng of pGEM 48 ~ 3.5 ul
     Need 2000 ng of pGEM 48 because we're using it twice, plates #5 and #6. The concentration of pGEM 48 is 579.5 ng/ul
  2. Added 1 ul of 10X CutSmart Buffer
  3. Added 4.5 ul Water
  4. Added 1.0 ul pMEI
  5. Vortexed

6. Incubated at 37'C for 2 hours

ssDNA Boiling:

1. Boil 10uL of 10mg/uL (per rxn) ssDNA in Thermocycler at 99'C for 10 mins
2. Immediately place on ice once finished for 10 mins

Yeast Transformation Protocol:

1. Transfer mixture from O/N Culture Dilution to new culturing tubes
2. Centrifuge at 3000 for 4 mins
3. Discard Supernatent
4. Resuspend cells with 1mL 0.1M LiOAc
5. Transfer over to 1.5mL tubes
6. Centrifuge cells at 3000 for 4 mins
7. Discard Supernatent
8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture
   -If Necessary, split into 100uL tubes for each transformation
9. Add 100ug (10uL) ssDNA to each 100uL sample
10. Add 5uL of target DNA
    -1:
        -pGEM 50
    -2:
        -pGEM 50
        -pGEM 48
11. Add in order:
    a. 480uL 50% PEG 3350
    b. 60uL 10X TE
    c. 60uL 1M LiOAc
    d. 75uL DMSO
12. Vortex
13. Incubate ar 42'C for 20 mins
14. Warm Selection Plates
    -1:
        -LEU2
    -2:
        -TRP + LEU2
15. Centrifuge tubes at 6000rpm for 2 mins
16. Discard Supernatent by pipetting
17. Resuspend in 500uL YPD
18. Centrifuge tubes at 6000rpm for 2 mins
19. Discard most of the Supernatent, leaving ~ 50uL
20. Resuspend cells in residual YPD
21. Plate on selective media
22. Incubate for 1-3 days at 30'C

PCR Purification for JS and EA

Samples need to purify

1. 20 ul

Protocol

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix
   a. 100 ul of Buffer PB
2. Check mixture that it is a yellow color

3. Pour supernatant into a spin column and centrifuge for 1 min to bind the DNA
4. Discard the flow through
5. To wash, add 0.75 ml of Buffer PE to the column and centrifuge for 1 min
6. Discard flow through & dry spin
7. Place the column in a 1.5 ml microcentrifuge tube
8. To elute DNA, add 50 ul of water to the center of the column
9. Let it stand 1 min then centrifuge it for 1 min

Nanodrop after PCR Purification

pGEM 49 Not1	30.54 ng/ul
pGEM 53 Sbf1	11.01 ng/ul

August 3rd, 2015

Yeast Colony PCR

1. Pick a single colony using a sterile pipette tip and mix in 25 ul of water in a PCR tube. Do this for about 4-6 colonies. Use 5 ul for the PCR reaction below, and save the rest for later.
2. Set up PCR reaction as below:

	1X	7X
2X Go Taq Green PCR Master Mix	10 ul	70ul
10uM FW Primer	1 ul	7 ul
10uM REV Primer	1 ul	7 ul
Water	3 ul	21 ul
Bacterial cells (template)	5 ul	35 ul

15 ul of the Master Mix goes into each 5 ul PCR tube

Protocol

30X		
	95	45s
	55	30s
	72	1 min per kb
72		10 min

3. Run it on a 1% agarose gel. GoTaq mix already has gel loading dye in it, so you can just directly load 5 ul of your PCR reaction into a gel.
4. For all positive bands on the gel, take the rest of the bacterial cells from step 1 and inoculate them into an O/N LB (+antibiotic) for miniprep the next day.

Dilute primers for Yeast Colony PCR

Mix 90 ul of water with 10 ul of the primer by vortexing.

Yeast Colony PCR Gel

1% Agarose Gel @ 100 volts for 30 mins

Key:

1. 2 Log DNA Ladder	10 ul
2. Yeast Colony PCR #1	5 ul
3. Yeast Colony PCR #2	5 ul
4. Yeast Colony PCR #3	5 ul
5. Yeast Colony PCR #4	5 ul
6. Yeast Colony PCR #5	5 ul
7. Yeast Colony PCR #6	5 ul
8. Blank	
9. 2 Log DNA Ladder	10 ul

10. Blank
11. JN + EC Sample #1 10 ul
12. JN + EC Sample #2 10 ul
13. JN + EC Sample #3 10 ul
14. JN + EC Sample #4 10 ul
15. JN + EC Sample #5 10 ul

See gel photo in box as "Yeast Colony PCR LexAOps+GFP"

yGEM 131 (LexAOps + GFP in yeast)

Liquid Culture of Yeast Colony PCR #5 and #6

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add 20 ul of our colony to PCR tubes into falcon tubes
3. Vortex
4. Place in 30'C spinner incubator o/n

August 4th, 2015

Yeast Transformation

Dilute yGEM 131 O/N culture

We want our culture to be in exponential phase so we do a 1:20 dilution in media

1. Add 9.5 ml of YPD
2. Add 500 ul of O/N culture
3. Incubate @ 30'C on the spinner for 3 hours

pGEM 48 Digestion:

1. Added 2000ng of pGEM50 ~ 3.4uL
2. Added 2uL CutSmart Buffer
3. Added 13.6uL Water
4. Added 1.0uL pMEI
5. Vortexed
6. Incubated at 37'C for 2 hours

ssDNA Boiling:

1. Boil 10uL of 10mg/uL (per rxn) ssDNA in Thermocycler at 99'C for 10 mins
2. Immediately place on ice once finished for 10 mins

OR

1. Boil ssDNA on a sandblock for 100'C for 10 mins
2. Place on ice immediately for 10 mins

Yeast Transformation Protocol:

1. Transfer mixture from O/N Culture Dilution to new culturing tubes
2. Centrifuge at 3000 for 4 mins
3. Discard Supernatent
4. Resuspend cells with 1mL 0.1M LiOAc
5. Transfer over to 1.5mL tubes
6. Centrifuge cells at 3000 for 4 mins
7. Discard Supernatent

```
        8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture
            -If Necessary, split into 100uL tubes for each transformation
        9. Add 100ug (10uL) ssDNA to each 100uL sample
        10. Add 5uL of target DNA
            -1:
                -pGEM48
        11. Add in order:
            a. 480uL 50% PEG 3350
            b. 60uL 10X TE
            c. 60uL 1M LiOAc
            d. 75uL DMSO
        12. Vortex
        13. Incubate ar 42'C for 20 mins
        14. Warm Selection Plates
            -1:
                -TRP
        15. Centrifuge tubes at 6000rpm for 2 mins
        16. Discard Supernatent by pipetting
        17. Resuspend in 500uL YPD
        18. Centrifuge tubes at 6000rpm for 2 mins
        19. Discard most of the Supernatent, leaving ~ 50uL
        20. Resuspend cells in residual YPD
        21. Plate on selective media
        22. Incubate for 1-3 days at 30'C
Streak Plates
    yGEM 131 #5
    yGEM 131 #6
Glycerol Stock of yGEM 131 #6
    Method #2
        In a cryovial:
            1. Add 350 ul of yeast cells
            2. Add 350 ul of 60% glycerol
            3. Vortex well for about 2 mins
            4. Dry ice to freeze and place in -80'C freezer
```

August 6th, 2015

```
Flow Cytometry
    Day Before
        Make Overnight cultures of strain to be tested in 5-10mL SD-Comp
    Flow Cytometry Prep Work
        1. Dilute overnight cultures 1:100 in 96 well shaker plate w/o beads
            a. Add 1000 ul SD-Comp media to all the wells
                -Use autorepeater to pipette in wells
            b. Add XuL Cell Culture
            c. Place back in foil
        2. Spectrophotometer:
            a. 900uL SD-Comp media per Cuvett
```

```
            b. 100uL O/N Culture
                -1:10 Dilution
            c. Read at 600nm
            d. Vortex all cuvetts, including blank
            e. Zero with blank first, then read other samples
        3. Calculate necessary sample volume
            - (Read * 10) / Desired OD (0.3) = Dilution Factor
            - Volume of Well (1000uL) / Dil-Factor = XuL per well for Sample
        4. Cover with breathable film
        5. Place in Incubator shaker for 3 hours
    Flow Cytometry Induce:
        1. 30mins prior to end of incubation, thaw necessary induction factor
        2. See "Flow" in google docs for dilution recipe
        3. Add 10uL of desire concentration of induction factor to each well
        4. Cover with breathable film
        5. Place in Incubator Shaker for 90mins - 2 hours, no more than 2 hrs
    Flow Cytometry Run:
        1. Transfer 250uL from each well to new 96 well V-Bottom Plate
        2. Add 10uL of cycloheximid to arrest cells
        3. Run on Flow Cytometer
        4. Analyze Data using Flojo
    **Running the Flow Cytometer**
        1. Check that machine is on standby and not run.
        2. Run a clean plate. (A1-A4 bleach, B1-B4 water)
        3. Check fluid buffer box and storage tanks.
        4. Make sure you have a positive and negative control so that you can           set the parameters.
        5. Open up FACSDiva. Sign into iGEM account.
        6. Create new FACS experiment. Highlight needed wells and use the blue          button to create wells
 for use. Create specimens among the wells              and rename them.
                a. Open up inspector to check parameters.
                b. Run well. (Do not click 'Run Plate'.)
                c. Once finished, export data onto a USB.
                d. Run a clean plate.
Liquid Culture
    1. yGEM 129
    2. yGEM 130
    3. yGEM 127
    4. CB008DB
        Under a flame:
            1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
            2. Add 20 ul of our colony to PCR tubes into falcon tubes
            3. Vortex
            4. Place in 37'C incubator o/n
```

August 7th, 2015

```
Yeast Transformation
    Dilute yGEM 127, yGEM 129, yGEM 130, CB008DB O/N culture
```

We want our culture to be in exponential phase so we do a 1:20 dilution in media

Error: Accidently put yGEM 129 into yGEM 127 liquid culture

1. yGEM 127

Add 5 ml of YPD and 250 ul of liquid culture (day before)

2. yGEM 129
3. yGEM 130
4. CB008DB
    a. Add 9.5 ml of YPD
    b. Add 500 ul of O/N culture
    c. Incubate @ 30'C on the spinner for 3 hours

pGEM 53 Digestion:

1. Added 4000ng of pGEM 53 ~ 17 ul

    The concentration was 234.8 ng/ul
2. Added 2 ul of 10X CutSmart Buffer
3. Added 1.0uL pMEI
4. Vortexed
5. Incubated at 37'C for 2 hours

ssDNA Boiling:

1. Boil 10uL of 10mg/uL (per rxn) ssDNA in Thermocycler at 99'C for 10 mins
2. Immediately place on ice once finished for 10 mins

OR

1. Boil ssDNA on a sandblock for 100'C for 10 mins
2. Place on ice immediately for 10 mins

Yeast Transformation Protocol:

1. Transfer mixture from O/N Culture Dilution to new culturing tubes
2. Centrifuge at 3000 for 4 mins
3. Discard Supernatent
4. Resuspend cells with 1mL 0.1M LiOAc
5. Transfer over to 1.5mL tubes
6. Centrifuge cells at 3000 for 4 mins
7. Discard Supernatent
8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture

    -If Necessary, split into 100uL tubes for each transformation
9. Add 100ug (10uL) ssDNA to each 100uL sample
10. Add 5uL of target DNA
    a. pGEM 53
11. Add in order:
    a. 480uL 50% PEG 3350
    b. 60uL 10X TE
    c. 60uL 1M LiOAc
    d. 75uL DMSO
12. Vortex
13. Incubate ar 42'C for 20 mins
14. Warm Selection Plates
    a. TRP
15. Centrifuge tubes at 6000rpm for 2 mins
16. Discard Supernatent by pipetting
17. Resuspend in 500uL YPD

18. Centrifuge tubes at 6000rpm for 2 mins
19. Discard most of the Supernatent, leaving ~ 50uL
20. Resuspend cells in residual YPD
21. Plate on selective media
22. Incubate for 1-3 days at 30'C

August 10th, 2015

Make more working stock of zymolase

1. Add 12 ul of zymolase
2. Add 988 ul of water

Yeast Colony PCR

Since we want to keep some of the O/N liquid culture, so we added culture to the PCR strip + 20 ul of water. We need to take out 5 ul of the cell + water and put it into a PCR strip with zymolyaze (50 ul) and pipette up & down

1. Pick a single colony using a sterile pipette tip and mix in 25 ul of water in a PCR tube. Do this for about 4-6 colonies. Use 5 ul for the PCR reaction below, and save the rest for later.
2. Set up PCR reaction as below:

	1X	13X
2X Go Taq Green PCR Master Mix	10 ul	130 ul
10uM FW Primer	1 ul	13 ul
10uM REV Primer	1 ul	13 ul
Water	3 ul	39 ul
Boiled yeast cells (template)	5 ul	65 ul

15 ul of the Master Mix goes into each 5 ul PCR tube

Protocol

30X		
	95	45s
	55	30s
	72	1 min per kb
72		10 min

3. Run it on a 1% agarose gel. GoTaq mix already has gel loading dye in it, so you can just directly load 5 ul of your PCR reaction into a gel.
4. For all positive bands on the gel, take the rest of the bacterial cells from step 1 and inoculate them into an O/N LB (+antibiotic) for miniprep the next day.

Colony PCR Gel

1.5% Agarose Gel

Key:

1. Ladder	10 ul
2. Blank	
3. yGEM 129 + pGEM 53 #1	5 ul
4. yGEM 129 + pGEM 53 #2	5 ul
5. yGEM 129 + pGEM 53 #3	5 ul
6. yGEM 129 + pGEM 53 #4	5 ul
7. yGEM 129 + pGEM 53 #5	5 ul
8. yGEM 129 + pGEM 53 #6 (Error: it solified)	
9. Blank	
10. Ladder	10 ul

11. Blank
12. yGEM 130 + pGEM 53 #1 5 ul
13. yGEM 130 + pGEM 53 #2 5 ul
14. yGEM 130 + pGEM 53 #3 5 ul
15. yGEM 130 + pGEM 53 #4 5 ul
16. yGEM 130 + pGEM 53 #5 5 ul
17. yGEM 130 + pGEM 53 #6 5 ul
18. Blank
19. Ladder 10 ul
20. Blank

Gel photo in box as "Yeast Colony PCR yGEM129+130 + pGEM53 #1"

August 11th, 2015

Yeast Colony PCR for yGEM 131 + pGEM 48 (For Jeffrey)

1. Pick a single colony using a sterile pipette tip and mix in 25 ul of water in a PCR tube. Do this for about 4-6 colonies. Use 5 ul for the PCR reaction below, and save the rest for later.
2. Set up PCR reaction as below:

	1X	7X
2X Go Taq Green PCR Master Mix	10 ul	70ul
10uM FW Primer	1 ul	7 ul
10uM REV Primer	1 ul	7 ul
Water	3 ul	21 ul
Bacterial cells (template)	5 ul	35 ul

15 ul of the Master Mix goes into each 5 ul PCR tube

Protocol

30X		
	95	45s
	55	30s
	72	1 min per kb
72		10 min

3. Run it on a 1% agarose gel. GoTaq mix already has gel loading dye in it, so you can just directly load 5 ul of your PCR reaction into a gel.
4. For all positive bands on the gel, take the rest of the bacterial cells from step 1 and inoculate them into an O/N LB (+antibiotic) for miniprep the next day.

Yeast Colony PCR for yGEM 129 + pGEM 53 & yGEM 130 + pGEM 53

1. Pick a single colony using a sterile pipette tip and mix in 25 ul of water in a PCR tube. Do this for about 4-6 colonies. Use 5 ul for the PCR reaction below, and save the rest for later.
2. Set up PCR reaction as below:

	1X	13X
2X Go Taq Green PCR Master Mix	10 ul	130ul
10uM FW Primer -161 (pNH605)	1 ul	13 ul
10uM REV Primer -87 (600 Series)	1 ul	13 ul
Water	3 ul	39 ul
Bacterial cells (template)	5 ul	35 ul

Added 15uL of Master Mix to 6 new PCR Tubes

Transferred 5uL from boiled Yeast cells PCR tubes to associated tube

Vortexed and centrifuged

Protocol

30X

	95	45s
	55	30s
	72	1 min per kb
72		10 min

3. Run it on a 1% agarose gel. GoTaq mix already has gel loading dye in it, so you can just directly load 5 ul of your PCR reaction into a gel.

4. For all positive bands on the gel, take the rest of the bacterial cells from step 1 and inoculate them into an O/N LB (+antibiotic) for miniprep the next day.

Colony PCR of yGEM 129 & yGEM 130 Gel

1.5% Agarose Gel (85 ml + 8.5 ul Sybersafe)

Key:

1. 2 Log DNA Ladder	10 ul
2. yGEM 129 Colony #1	5 ul
3. yGEM 129 Colony #2	5 ul
4. yGEM 129 Colony #3	5 ul
5. yGEM 129 Colony #4	5 ul
6. yGEM 129 Colony #5	5 ul
7. yGEM 129 Colony #6	5 ul
8. Blank	
9. 2 Log DNA Ladder	10 ul
10. Blank	
11. yGEM 130 Colony #1	5 ul
12. yGEM 130 Colony #2	5 ul
13. yGEM 130 Colony #3	5 ul
14. yGEM 130 Colony #4	5 ul
15. yGEM 130 Colony #5	5 ul
16. yGEM 130 Colony #6	5 ul
17. Blank	
18. 2 Log DNA Ladder	10 ul

See gel photo in box "Yeast Colony PCR yGEM129+130 + pGEM53 #2"

Colony PCR of yGEM 131 + pGEM 48 Gel

1.5% Agarose Gel (35 ml + 3.5 ul Sybersafe)

Key:

1. Skip	
2. Ladder	10 ul
3. Blank	
4. yGEM 131 + pGEM 48 Colony #1	5 ul
5. yGEM 131 + pGEM 48 Colony #2	5 ul
6. yGEM 131 + pGEM 48 Colony #3	5 ul
7. yGEM 131 + pGEM 48 Colony #4	5 ul
8. yGEM 131 + pGEM 48 Colony #5	5 ul
9. yGEM 131 + pGEM 48 Colony #6	5 ul
10. yGEM 131 + pGEM 48 Colony #7	5 ul
11. yGEM 131 + pGEM 48 Colony #8	5 ul
12. Blank	
13. Ladder	10 ul

14. Ladder Leaked
See gel photo in box "Yeast Colony PCR yGEM131 + pGEM48"
Liquid Culture
1. yGEM 129 #3
2. yGEM 130 #6
3. yGEM 131 #8
Under a flame:
1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add 20 ul of our colony to PCR tubes into falcon tubes
3. Vortex
4. Place in 37'C incubator o/n

August 12th, 2015

Yeast Transformation
Dilute yGEM 133, yGEM 136, yGEM 137 O/N culture
We want our culture to be in exponential phase so we do a 1:20 dilution in media
Error: Accidently put yGEM 129 into yGEM 127 liquid culture
1. yGEM 127
Add 5 ml of YPD and 250 ul of liquid culture (day before)
2. yGEM 129
3. yGEM 130
4. CB008DB
a. Add 9.5 ml of YPD
b. Add 500 ul of O/N culture
c. Incubate @ 30'C on the spinner for 3 hours
pGEM 53 Digestion:
1. Added 4000ng of pGEM 53 ~ 17 ul
The concentration was 234.8 ng/ul
2. Added 2 ul of 10X CutSmart Buffer
3. Added 1.0uL pMEI
4. Vortexed
5. Incubated at 37'C for 2 hours
ssDNA Boiling:
1. Boil 10uL of 10mg/uL (per rxn) ssDNA in Thermocycler at 99'C for 10 mins
2. Immediately place on ice once finished for 10 mins
OR
1. Boil ssDNA on a sandblock for 100'C for 10 mins
2. Place on ice immediately for 10 mins
Yeast Transformation Protocol:
1. Transfer mixture from O/N Culture Dilution to new culturing tubes
2. Centrifuge at 3000 for 4 mins
3. Discard Supernatent
4. Resuspend cells with 1mL 0.1M LiOAc
5. Transfer over to 1.5mL tubes
6. Centrifuge cells at 3000 for 4 mins
7. Discard Supernatent
8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture

-If Necessary, split into 100uL tubes for each transformation
9. Add 100ug (10uL) ssDNA to each 100uL sample
10. Add 5uL of target DNA
a. pGEM 53
11. Add in order:
a. 480uL 50% PEG 3350
b. 60uL 10X TE
c. 60uL 1M LiOAc
d. 75uL DMSO
12. Vortex
13. Incubate ar 42'C for 20 mins
14. Warm Selection Plates
a. TRP
15. Centrifuge tubes at 6000rpm for 2 mins
16. Discard Supernatent by pipetting
17. Resuspend in 500uL YPD
18. Centrifuge tubes at 6000rpm for 2 mins
19. Discard most of the Supernatent, leaving ~ 50uL
20. Resuspend cells in residual YPD
21. Plate on selective media
22. Incubate for 1-3 days at 30'C

There was very little amounts of cells for all of the yGEMs. The outcome of the transformation will probably be low so the we will redo it tomorrow

Glycerol Stock of yGEM 136, yGEM 137, yGEM 138

1. Labeled a cyrovial
2. Added 350uL of 60% glycerol
3. Added 350uL of O/N culture
4. Vortexed for 2 mins
5. Freeze with dry ice for 2 mins
6. Store in -80'C freezer

Yeast Liquid Culture

1. yGEM 136
2. yGEM 137
3. yGEM 138
4. CB008DB

Under a flame:

1. Add 5 mL of LB + Carb media into 5 falcon tubes (colonies 1-5)
2. Add 20 ul of our colony to PCR tubes into falcon tubes
3. Vortex
4. Place in 37'C incubator o/n

August 13th, 2015

Re-did the Yeast Transformations for yGEM 136 and yGEM 137

pGEM48 Digestion

1. Added 2000ng of pGEM48 ~ 4uL
2. Added 1uL CutSmart Buffer
3. Added 1.0uL pMEI

4. Added 4.0uL Water
5. Vortexed
6. Incubated at 37'C for 2 hours

O/N Culture Dilution:

Dilution of 1:20 in YPD

1. 250uL of O/N Cultures
2. 4.75mL YPD
3. Incubated at 30'C with spinning for 3 hours

ssDNA Boiling:

1. Boil 10 ul of 10mg/ul (per rxn) ssDNA in Thermocycler at 99'C for 10mins
2. Immediately place on ice once finished for 10 mins

Yeast Transformation Protocol:

1. Transfer mixture from O/N Culture Dilution to new culturing tubes
2. Centrifuge at 3000 for 4 mins
3. Discard Supernatent
4. Resuspend cells with 1mL 0.1M LiOAc
5. Transfer over to 1.5mL tubes
6. Centrifuge cells at 3000 for 4 mins
7. Discard Supernatent
8. Resuspend cells with 100uL 0.1M LiOAc per 2.5mL Culture
   -If Necessary, split into 100uL tubes for each transformation
9. Add 100ug (10uL) ssDNA to each 100uL sample
10. Add 5uL of target DNA
    a. pGEM 53
11. Add in order:
    a. 480uL 50% PEG 3350
    b. 60uL 10X TE
    c. 60uL 1M LiOAc
    d. 75uL DMSO
12. Vortex
13. Incubate ar 42'C for 20 mins
14. Warm Selection Plates
    a. TRP
15. Centrifuge tubes at 6000rpm for 2 mins
16. Discard Supernatent by pipetting
17. Resuspend in 500uL YPD
18. Centrifuge tubes at 6000rpm for 2 mins
19. Discard most of the Supernatent, leaving ~ 50uL
20. Resuspend cells in residual YPD
21. Plate on selective media
22. Incubate for 1-3 days at 30'C

Flow Cytometery yGEM138

Flow Cytometry Prep Work

Dilute overnight cultures 1:100 in 96 well shaker plate

1. 1000uL SD-Comp
2. XuL Cell Culture
   -See Spectrophotometer

Spectrophotometer:

1. 900uL SD-Comp per Cuvett
2. 100uL O/N Culture
    -1:10 Dilution
3. Read at 600nm
4. Vortex all cuvetts, including blank
5. Zero with blank first, then read other samples
6. Calculate necessary sample volume
    - (Read * 10) / Desired OD (0.3) = Dilution Factor
    - Volume of Well (1000uL)/Dil-Factor = XuL per well for Sample
7. Cover with breathable film
8. Place in Incubator shaker for 3 hours

Flow Cytometry Induce:

1. 30mins prior to end of incubation, thaw necessary induction factor
2. See "Flow" in google docs for dilution recipe
3. Add 10uL of desire concentration of induction factor to each well
4. Cover with breathable film
5. Place in Incubator Shaker for 90mins - 2 hours

Flow Cytometry Run:

1. Transfer 250uL from each well to new 96 well V-Bottom Plate
2. Add 10uL of cycloheximid to arrest cells
3. Run on Flow Cytometer
4. Analyze Data

August 14th, 2015

Jeffrey's Prep work for Flow

DOX Dilutions

Stock: 50 mg/mL

1x	**100x**	Prep
60µg/mL	A: 6mg/mL	60µL of Stock in µL 440µL of water
30µg/mL	B: 3mg/mL	30µL of Stock in 470µL of water
9µg/mL	C: 900µg/mL	75 µL of A in 425µL of water
6µg/mL	D: 600µg/mL	50 µL of A in 450 µL of water
3µg/mL	E: 300µg/mL	25 µL of A in 475 µL of water
0.9µg/mL	F: 90µg/mL	50 µL of C in 450µL of water
0.6µg/mL	G: 60µg/mL	50 µL of D in 450µL of water
0.3µg/mL	H: 30µg/mL	50 µL of E in 450µL of water
0.09µg/mL	I: 9µg/mL	50 µL of F in 450µL of water
0.06µg/mL	J: 6µg/mL	50 µL of G in 450µL of water
0.03µg/mL	K: 3µg/mL	50 µL of H in 450µL of water